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ISOLATION AND MOLECULAR CHARACTERIZATION OF MICROORGANISMS WITH BIOFERTILIZER POTENTIAL

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ABSTRACT

Biofertilizers are microbial-agro products containing mixed culture of microorganisms that promote plant growth, yield, soil quality and disease control. This study aimed to isolate, identify and screen microorganisms with biofertilizer potentials for application in farms. Soil samples were collected from farmland and waste-dump soils around University of Port Harcourt. The various microorganisms were isolated and estimated using nutrient agar, potato dextrose agar, cetrimide agar and Ashby's agar. The microorganisms were screened for biofertilizer potentials based on nitrogen fixation, potassium and phosphate solubilization using Pikovskaya media. The results obtained from this study showed that thefarmland soil sample had a total heterotrophic bacterial and fungal counts of 5.045±0.02 and 4.220±0.02 Log_{10}Cfu/gwhile the corresponding values in the waste-dump soil was 4.890±0.30 and 3.505±0.30 Log_{10}Cfu/g respectively. After screening, the microorganisms with biofertilizer potentials were identified as Aspergillus niger, Penicillium chrysogenum, Bacillus cereus, Bacillus licheniformis, Pseudomonas fluorescens and Azotobacter chroococcum. Findings from this study have demonstrated that the microorganisms isolated from the farmland soil were more adept at nitrogen fixation and solubilizing insoluble potassium and phosphate compounds than their counterparts in waste-dump soil. These microorganisms have shown potentials to improve soil fertility and crop productivity in a sustainable way.

Keywords: Biofertilizer, microorganisms, plant growth, nitrogen fixation, phosphate solubilization

INTRODUCTION

Biofertilizer application has been strongly advocated as the best substitute of chemical fertilizer use because biofertilizers are cheap, effective and environmentally friendly agro-products that enhance plant growth, yield and soil quality (Ammar *et al.*, 2023).Globally, it is recognized as an important component of the integrated nutrient supply management system and hold a great promise to enhance crop yields through environmentally better nutrient supplies (Wu *et al.*, 2005). Biofertilizers improve root proliferation due to the release of growth promoting hormones and convert complex nutrients into simple nutrients thereby making them available to plants (Ammar *et al.*, 2023). Biofertilizers are products of one or more species of microorganisms which have the ability to mobilize nutritionally important elements from non-useable to useable forms through biological processes such as nitrogen fixation, phosphate solubilization, excretion of plant growth promoting substances and

biodegradation in soil. Biofertilizers are living microbial inoculants of bacteria, algae, fungi alone or in combination. The role of biofertilizers in agriculture assumes special significance, particularly in the present context of high cost of chemical fertilizers and their hazardous effects on environmental health (Kumar *et al*., 2017).

The development and use of microbial-based fertilizers have recently gained significance due to the recognition of the deleterious effects on the environment generated by the excessive and improper application of chemical fertilizers (Maurya *et al*., 2014). This was a result of the improved knowledge about the relationships occurring in the rhizosphere, between the plant and all soil microorganisms, as well as due to the immense efforts in isolating and selecting microbial strains showing plant growth promoting capabilities (Parmar and Sindhu, 2013).

Soil microorganisms have been used in crop production for many years (Hayat *et al*., 2010). Microbes in the soil are directly tied to nutrient recycling especially carbon, nitrogen, phosphorus and sulfur and bacteria are the major class of microorganisms that keep soils healthy and productive (Thornbro, 2022). Some of the main functions of bacteria in the soil include: supply of nutrients to the crops, enhancement of plant growth, control of activities of plant pathogens and improvement of soil structure and quality. Effective microorganisms can also be used for bioremediation in polluted soils (Hayat *et al*., 2010; Atuchin *et al.,* 2023).

Some of the microorganisms used as biofertilizers include bacteria (*Rhizobium, Bradyrhizobium, Azospirillum, Azobacter, Bacillus,* and *Pseudomonas* species) and fungal species such as mycorrhizal fungi, *Aspergillus Penicillium*, *Chaetomium* and *Trichoderma* (Kaechai and Hyde, 2009; Seenivasagan *et al*., 2021).Microbial mechanisms of plant growth promotion include biological nitrogen fixation (BNF), synthesis of phytohormones, environmental stress relief, synergism with other microbial-plant interactions, inhibition of plant ethylene synthesis, as well as increasing availability of nutrients like phosphorus, iron and minor elements, and growth enhancement by volatile compounds (Ammar *et al.,* 2023). However, the expression of such bacterial activities under laboratory conditions does not guarantee in mutual or symbiotic association with a host plant. This is especially true of nitrogen fixation as abundantly expressed in culture media by many bacterial and fungal species. The mechanisms of plant growth promotion have been analyzed in different organisms (Paul and Dubey, 2014).

Beside their roles on the availability of nutrients, soil microorganisms prevent the uptake of several harmful ions. The use of living microbial cells (biofertilizer) accelerates mineralization of organic residues in soil, therefore making the complex nutrients more available to crops. Thus, this study was designed to screen, isolate, and identify microorganisms with biofertilizer potentials of fixing nitrogen and solubilizing phosphate and potassium compounds.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected from farmland and waste-dump soils of University of Port Harcourt, Nigeria with the aid of a hand-held soil auger using the method adopted by Uzah *et al*. (2020). The soil samples were randomly collected from different depth between 0 to l5cm and then bulked together to obtain a composite soil. The hand-held soil auger used was cleansed after each collection to reduce contamination between samples. The soil samples were kept in a sterile polythene bag and conveyed on ice pack to the laboratory for analysis.

Isolation of Microorganisms from the Soil Samples

A tenfold serial dilution was performed on the soil samples in accordance with the description by Jalal *et al*. (2010). Nine (9) millilitres of normal saline (0.85% NaCl w/v

Scientia Africana, Vol. 23 (No. 1), February, 2024. Pp 173-188
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in distilled water) were dispensed into each of the cleansed test tubes and 90 mL was dispensed into Erlenmeyer flasks, and sterilized in an autoclave at 121^0C for 15min at 15psi and thereafter allowed to cool. The soil samples were carefully homogenized separately and 10g of each homogenized sample was introduced into the 250 mL Erlenmeyer flasks and made up to 100mL mark with the sterile normal saline to make a stock solution to obtain 1:10 dilution; from this stock solution several (10^{-1} – 10^{-6}) dilutions were made. Analiquot (0.1mL) from various dilutions (10^{-2}– 10^{-4}) were plated out in duplicate using spread plate method on potato dextrose agar (PDA) (containing antibiotic to suppress bacterial growth), nutrient agar, cetrimide agar and Ashby's agar and incubated at 28 ± 2°C for 2 – 7days accordingly. Morphologically distinct colonies were sub-cultured repeatedly until pure cultures were obtained. The isolates were stored on agar slant for further studies.

Cultural and Biochemical Characterization of the Isolates

The fungal and bacterial isolates were identified using their morphological and biochemical characteristics (Agu *et al.* 2021).

Screening for Nitrogen Fixation, Phosphorus and Potassium Solubilization Abilities

The method for the screening of microorganisms for biofertilizer potentiality as described by Agu *et al.* (2021) was adopted. The isolates were spot inoculated at the centre of prepared Pikovskayamedium for phosphate solubilization; Aleksandrov medium for potassium solubilization and the inoculated plates were incubated for 72 h at 30^0C. The formation of clear zones around the colonies indicate phosphate and potassium solubilization, respectively. The nitrogen fixing ability of the isolates were assessed using glucose nitrogen free mineral medium (GNFM) and the ability of the isolates to fix N_2 was observed by the change in colour of the medium after the incubation period to blue. The solubilization ability of the isolates was determined by measuring the solubilization index. The medium without the inoculants was used as control.

Molecular Identification

Fungal and bacterial isolates capable of fixing nitrogen, and solubilizing potassium and phosphate were further identified and characterized using molecular methods which involves extracting the DNA, PCR amplification of the fungi 18S and bacteria 16S rRNA and gel electrophoresis of the isolates at the Biotechnology Research Centre, University of Port Harcourt. 18S and 16S rRNA sequencing was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan (Uzah *et al.* 2020;Guardiola-Márquez *et al.*, 2023).

RESULTS

The population of various microbial groups in the soil samples are as presented in Table 1. The highest total heterotrophic bacterial count (THBC) of 5.045±0.02Log_{10} CFU/g was obtained in farmland soil while waste-dump soil had the lowest count of 4.890±0.30Log_{10} CFU/g. Total fungal count (TFC) was also high (4.220±0.02 Log_{10} CFU/g) in farmland soil when compared to waste-dump soil (3.505±0.30Log_{10} CFU/g). *Pseudomonas* and *Azotobacter* counts of 4.725±0.02 Log_{10} CFU/g and 4.805±0.02 Log_{10} CFU/g respectively were obtained in farmland soil while in waste-dump soil, lower counts of 4.650±0.30 Log_{10} CFU/g and 4.505±0.30 Log_{10} CFU/gwere obtained for *Pseudomonas* and *Azotobacter* respectively. However, no significant difference in counts for each microbial group was obtained between the two soils at $P \leq .0.245$.

The cultural characterization of fungal isolates based on their colonial morphology and microscopic features are presented in Table 2. The fungal species identified were *Aspergillus niger, Aspergillus flavus, Penicillium* sp, *Rhizopus* sp. *and Mucor* sp. Table 3 presents the microscopic and biochemical test result of the bacterial isolates. The bacterial species identified were *Pseudomonas fluorescens,*

Pseudomonas aeruginosa, Paraburkholderia oxyphilia, Bacillus sp., *Azotobacter* sp. and *Staphylococcus* sp.

Table 4 presents the plant promoting abilities of the isolates. The results indicate that 42.31% of the bacterial isolates possess the ability to fix nitrogen whereas, no fungal isolate could fix nitrogen. The number of isolates which had the potential to solubilize potassium was 73.08 % while 65.38% solubilized phosphate. The results obtained indicate that all the *Azotobacter* species isolated were nitrogen fixers. Meanwhile, the isolates from farmland soil demonstrated better capabilities at promoting plant growth than isolates obtained from waste-dump soil.

Figures 1 – 4show the molecular characteristics and identification of the selected fungal and bacterial isolates. The18S rRNA and 16S rRNA sequences obtained from the fungal and bacterial isolates produced an exact match during the mega blast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database respectively. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 18S rRNA of the isolate (FF3) -AN within the *Aspergillus niger*. Isolate (FF4) – PS was found to be closely related to *Penicillium chrysogenum*(Figure 2) (Jukes and Cantor, 1969; Saitou and Nei, 1987).

The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates (FB5)-BS1, (FB9)-BS2, (FB1) -PF and (FB13)-AZ revealed a closely relatedness to *Bacillus cereus, Bacillus licheniformis, Pseudomonas fluorescens* and *Azotobacter chroococcum* respectively (Figure 4). The 18S and 16S rRNA of the isolates showed a percentage similarity to other species at 99 -100% (Table 5). (Jukes and Cantor, 1969; Saitou and Nei, 1987).

Results of the 1% agarose gel electrophoresis for the visualization of the 18S and 16S rRNA region of the rRNA gene of the isolates showed that the PCR products were amplified to possess a molecular weight that each corresponds to 500 and 1500 base pairs (bp) of fungal and bacterial gene, respectively. The purified lanes labeled AN and PS shown in Figure 2 represent the 18S rRNA gene bands (500bp), while lane L or MK represent the 100bp molecular ladder. Furthermore, the purified lanes labeled BS1, BS2, PF and AZ shown in Figure 4 represent the 16S rRNA gene bands (1500bp), while lane L or MK represent the 100 bp molecular ladder. Sequence identification of the screened fungal and bacterial isolates from NCBI BLAST hits and their percentage relatedness are shown in Table 5 which shows 99% relatedness.

Table 1:Colony Counts(Log_{10}CFU/g) of various microbial group in soil samples

Count Type	Soil Type	
	Farmland soil	**Waste-dump soil**
THBC	5.045±0.02 [a]	4.890±0.30 [a]
TAC	4.805±0.02 [b]	4.505±0.30 [a]
TPC	4.725±0.02 [b]	4.650±0.30 [a]
TFC	4.220±0.02 [c]	3.505±0.30 [b]
P-value	< 0.0001	0.105
Significant	Yes	No

KEYS:

THBC =Total Heterotrophic Bacterial Count
TAC = Total *Azotobacter* Count
TPC = Total *Pseudomonas* Count
TFC = Total Fungal Count

Scientia Africana, Vol. 23 (No. 1), February, 2024. Pp 173-188
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Table 2: Cultural Characterization of Fungal Isolates

Isolates	Macroscopy	Microscopy	Probable organisms
FF1	Green powdery surface surrounded by white lawn, brown reverse symmetry	Septate hyphae with septate conidiosphores bearing conidia	*Penicillium* sp.
FF2	Surface colony colour is Light green lawn surrounded by white lawn-like growth without radial symmetry	Septate hyphae with septate conidiophores bearing conidia	*Aspergillus flavus*
FF3	Growth rate is rapid and textures of colonies are powdery and produced radial tissues in the agar. Surface colony colour was initially white becoming deep brown with conidial production while the reverse is pale yellow.	Septate hyphae with globose and radiate conidia heads with metulae that support the phialides; conidiosphores are hyaline and smooth-walled	*Aspergillus niger*
FF4	Green powdery surface surrounded by white lawn, brown reverse symmetry	Septate hyphae with septateconidiosphores bearing conidia	*Penicillium* sp.
FF5	Growth rate is rapid with white cotton colonies and pale yellow symmetry	Sporangia are greyish-black, spherical with branching hyphae that lack cross-walls	*Rhizopus* sp.
WF1	Surface colony colour is Light green lawn surrounded by white lawn-like growth without radial symmetry	Septate hyphae with septate conidiophores bearing conidia	*Aspergillus flavus*
WF2	Growth rate is rapid and textures of colonies are powdery and produced radial tissues in the agar. Surface colony colour was initially white becoming deep brown with conidial production while the reverse is pale yellow.	Septate hyphae with globose and radiate conidia heads with metulae that support the phialides; conidiosphores are hyaline and smooth-walled	*Aspergilluniger*
WF3	Growth rate is rapid with white fluffy colonies and reverse white symmetry	Sporangia are greyish-black, spherical with branched sporangiophore, with no **rhizoids and stolons**	*Mucor* sp.

Key: FF= farmland soil, FW= waste dump soil,

Table 3: Microscopic and Biochemical Characterization of Bacterial Isolates obtained from farmlands and waste dump soil

Isolates Code	Gram Reaction	SHAPE	CAT	OXI	CIT	MOT	MR	VP	UREASE	IND	GLU	LAC	MAN	SUC	Suspected Organism
FB1	-Ve	Rods	+	+	+	+	-	-	+	-	-	-	-	-	*Pseudomonas fluorescens*
FB2	-Ve	Rods	+	+	+	+	-	-	-	-	+	-	+	-	*Pseudomonas aeruginosa*
FB3	-Ve	Rods	+	+	+	+	+	+	+	-	-	-	-	-	*Pseudomonas fluorescens*
FB4	-Ve	Rods	+	-	+	-	-	-	+	-	A	-	+	-	*Paraburkholderia oxyphilia*
FB5	+Ve	Rods	+	-	+	+	-	-	+	-	A	-	A	A	*Bacillus* sp
FB6	+Ve	Rods	+	-	+	-	-	-	+	-	A	-	-	A	*Bacillus* sp
FB7	+Ve	Rods	+	-	+	-	-	-	+	-	A	-	A	A	*Bacillus* sp
FB8	+Ve	Rods	+	-	+	+	+	+	+	-	A	-	A	-	*Bacillus* sp
FB9	+Ve	Rods	+	-	+	+	-	+	+	-	A	-	-	A	*Bacillus* sp
FB10	+Ve	Rods	+	+	+	+	-	+	+	-	A	-	-	A	*Azotobacter* sp
FB11	+Ve	Rods	+	-	+	+	-	-	+	-	A	-	-	A	*Azotobacter* sp
FB12	+Ve	Rods	+	-	+	+	-	-	+	-	A	-	A	A	*Azotobacter* sp
FB13	+Ve	Rods	+	-	+	+	+	+	+	-	A	-	A	A	*Azotobacter* sp
WB1	+Ve	Rods	+	-	+	-	-	-	+	-	A	-	A	A	*Bacillus* sp
WB2	+Ve	Rods	+	-	+	+	+	+	+	-	A	-	A	-	*Bacillus* sp
WB3	-Ve	Rods	+	+	+	+	-	-	-	-	+	-	+	-	*Pseudomonas aeruginosa*
WB4	+Ve	Rods	-	+	+	+	+	+	+	-	A	-	A	A	*Azotobacter* sp
WB5	+Ve	Cocci	+	-	+	-	+	+	+	-	+	+	+	+	*Staphylococcus* sp

KEY: FB= Farmland Soil Bacteria; WB= WasteDump Soil Bacteria

MOT = Motility, OXI = Oxidase, CAT = Catalase, IND = Indole Production, VP = Voges-Proskauer, MRT = Methyl Red Test, UR = Urease, SUC = Sucrose, GLU = Glucose, LAT = Lactose, MAN = Mannitol, CIT = Citrate A = Acid, AG = Acid /gas, + = Positive = Negative.

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Table 4: Screening of the Fungal and Bacterial Isolates for Plant growth Promoting Abilities

Isolate Codes	Probable Organisms	Nitrogen Fixing Ability	Potassium Solubilizing Ability	Phosphorus Solubilizing Ability
FB1	*Pseudomonas fluorescens*	+	+	+
FB2	*Pseudomonas aeruginosa*	-	-	+
FB3	*Pseudomonas fluorescens*	-	+	+
FB4	*Paraburkholderia oxyphilia*	+	-	-
FB5	*Bacillus spp.*	+	+	+
FB6	*Bacillus spp.*	-	-	-
FB7	*Bacillus spp.*	+	-	+
FB8	*Bacillus spp.*	-	+	+
FB9	*Bacillus spp.*	+	+	+
FB10	*Azotobacter spp.*	+	+	-
FB11	*Azotobacter spp.*	+	+	+
FB12	*Azotobacter spp.*	+	-	-
FB13	*Azotobacterspp.*	+	+	+
WB1	*Bacillus spp.*	+	+	-
WB2	*Bacillus spp.*	-	+	+
WB3	*Pseudomonas aeruginosa*	-	+	+
WB4	*Azotobacter spp.*	+	+	-
WB5	*Staphylococcus spp.*	-	-	+
FF1	*Penicillium spp.*	-	+	+
FF2	*Aspergillus flavus*	-	+	-
FF3	*Aspergillus niger*	-	+	+
FF4	*Penicillium spp.*	-	+	+
FF5	*Rhizopus spp.*	-	+	-
WF1	*Aspergillus flavus*	-	+	-
WF2	*Aspergillu niger*	-	-	+
WF3	*Mucor spp.*	-	+	+

KEY: FB= Farmland Soil Bacteria; WB= WasteDump Soil Bacteria
(+) = Positive Reaction; (-) = Negative Reaction

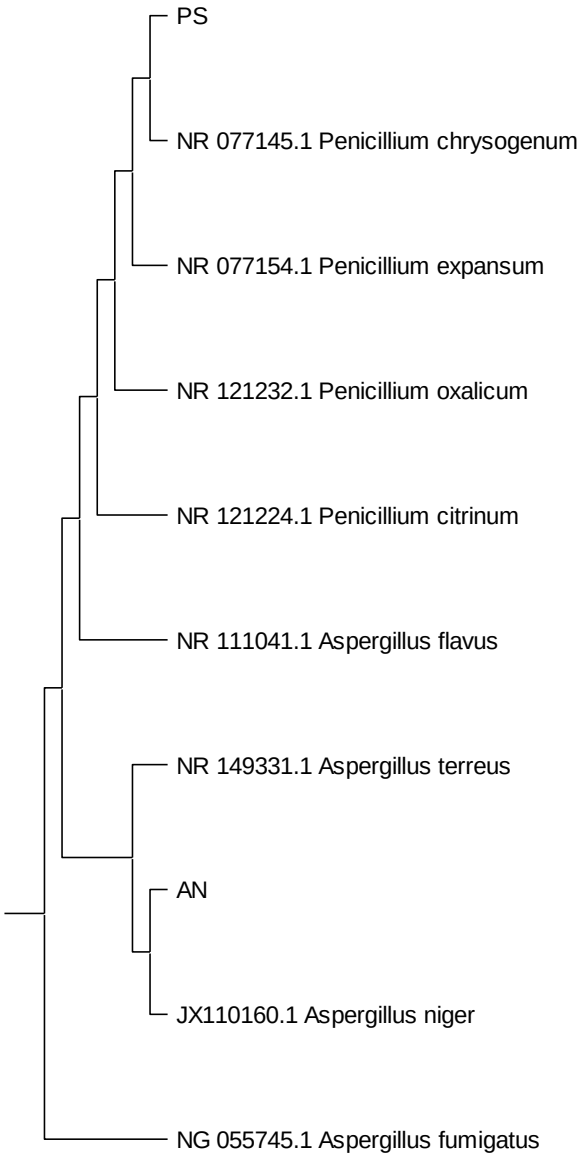

Fig. 1: Evolutionary Relationship of Fungal Isolates and their Closest Genbank Relative

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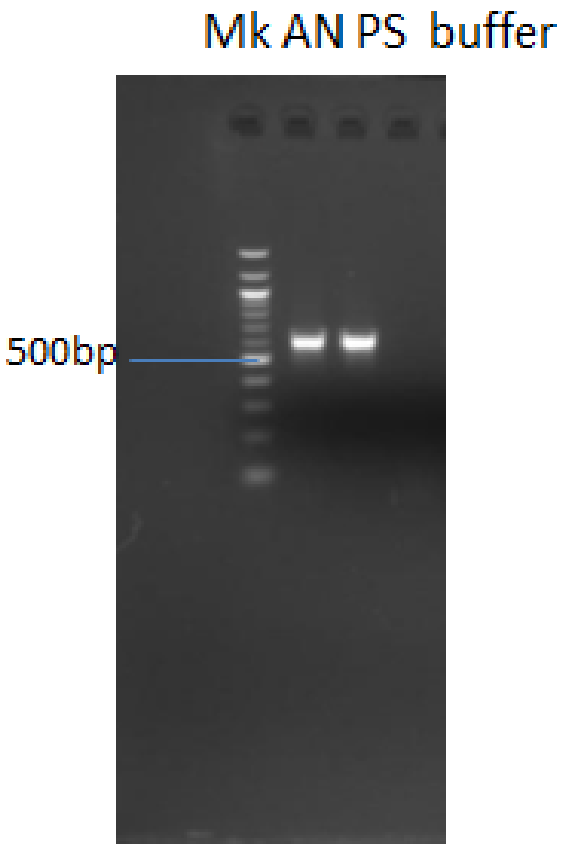

Fig 2: Agarose gel electrophoresis indicating the positive amplification of the `I8S region in the extracted fungi DNA

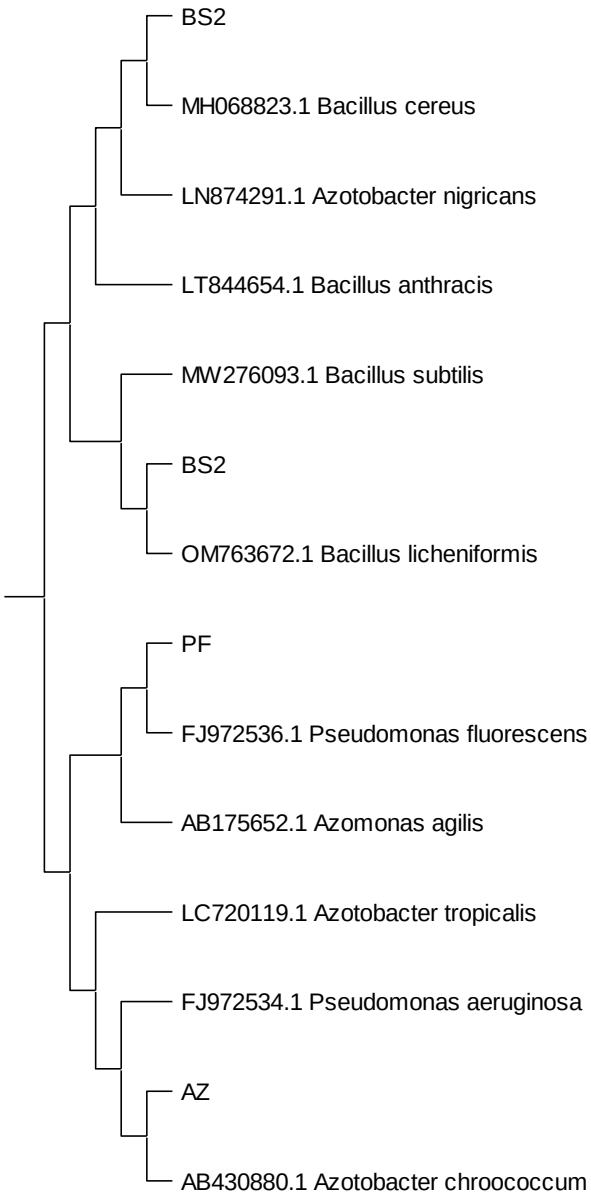

Fig. 3: Evolutionary Relationship of Bacterial Isolates and their Closest Genbank Relative

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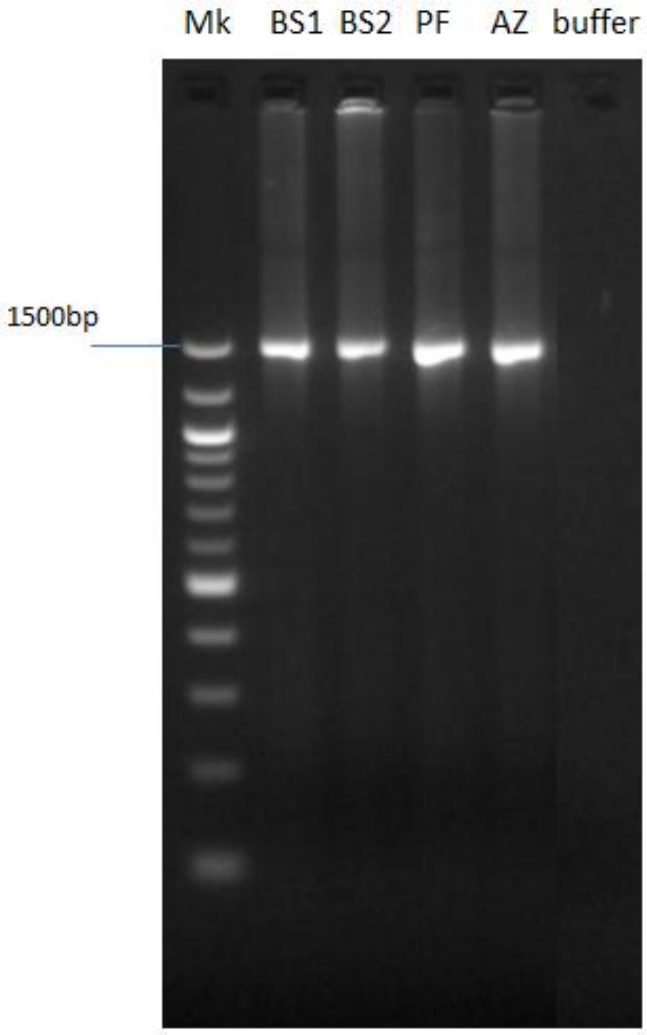

Fig 4: Agarose Gel Electrophloresis Indicating the Positive Amplification of The `16S rRNA Region in the Extracted Bacteria DNA

Table 5: Sequence Identification from NCBI BLAST hits and their Percentage Relatedness

Sample ID	Scientific Name	Maximum Score	Total Score	Query Cover	E value	Percent Identity	Accession number
BS2	*Bacillus licheniformis*	1840	1840	99%	0	99.90%	OP970169
PF	*Pseudomonas fluorescens*	2610	2610	99%	0	99.79%	OP970170
AZ	*Azotobacter chroococcum*	2603	2603	99%	0	99.79%	OP970171
BS1	*Bacillus cereus*	2656	37125	99%	0	99.72%	OP970172
AN	*Aspergillus niger*	1007	1007	99%	0	99.64%	OP970215
PS	*Penicillium chrysogenum*	1026	1026	99%	0	99.82%	OP970216

DISCUSSION

The microbial counts of soil samples (Table 1) obtained in this study ranged from 3.51 to 5.05 Log_{10}CFU/g. These counts were lower than microbial counts in soil according to the reports of Agu *et al*. (2021) and Paul and Dubey (2014),who obtained a range of 5.8 to $8.2x10^6$ CFU/g and 10^6 - 10^9 CFU/g, respectively. The results indicate that microbial counts of a soil sample is influenced by the type of soil, microbial growth conditions and media used for the cultivation. The results also demonstrated that farmland and waste-dump soil have essential microbes for biofertilizer and other bio-products production. According to Arsheen and Shailaja (2016), one way to increase crop yield is by using beneficial microorganisms. Biofertilizers help the crops to fix atmospheric nitrogen and make phosphate available for the plants. They maintain a healthy symbiotic relationship with the crops thus, helping to increase the yield of the crops. This symbiotic relationship proves beneficial to the organism by contributing to their sustenance which in turn increase the soil fertility, disease free and resistant crop type (Mohd and Taqi, 2014).

The fungal isolates identified in this study (Table 2) which include; *Aspergillus niger, Aspergillus flavus, Penicillium* sp., *Rhizopus* sp. *and Mucor sp.* and bacterial isolates (Table 3) is in agreement with the reports of Kaechai & Hyde, (2009); Sharma *et al*. (2011); and Amal and Heba (2023),who reported the following microorganisms as beneficial to pant growth. They include *Alcaligenes*, *Bacillus, Azotobacter, Enterobacter* and *Pseudomonas*; mycorrhizal fungi, *Penicillium*, *Chaetomium* and *Trichoderma.* In a similar study, Agu *et al*. (2021) reported *Pseudomonas fluorescens, Pseudomonas aeruginosa, Paraburkholderia oxyphilia, Bacillus* sp., *Azotobacter* sp., and *Staphylococcus* sp. to be beneficial for plant growth enhancement. In other words, these microorganisms could be used as a biofertilizer. Furthermore, farmland soils harbour beneficial microorganisms for plant growth enhancement due to the symbiotic relationships in the rhizosphere of plants (Arsheen and Shailaja, 2016). Low potentiality of the organisms isolated from the waste dump soil could be as result of discharge of heavy metals and other soil pollutants.

Bacterial species screened and identified in this study for the production of biofertilizer which include; *Bacillus cereus*(OP970172), *Bacillus licheniformis* (OP970169), *Pseudomonas fluorescens* (OP970170) and *Azotobacter chroococcum* (OP970171) have been proven to be useful in enhancing plant growth through mineralization such as solubilization of phosphate, potassium, calcium compounds and nitrogen fixation (Amal and Heba, 2023; Agu *et al*., 2021). At present, there is no doubt that, the legume tubercle bacteria (*Bacillus radicicola vars*.) and certain soil forms (notably *Azotobacter* spp. and *Clostridium Pasteurianrum*) possess the capacity to fix nitrogen. Cheng and Fanyu (2014) reported that potassium (K) is essential to plant growth and development; it helps in the utilization of nitrogen (N), and synthesis of protein and sugar. In plants, K deficiency is responsible for yellowing of the leaf edges and can also lead to slow growth and incomplete root development. Previous studies have shown that potassium solubilizing bacteria (KSB) can promote plant growth (Shanware *et al*., 2014; Agu *et al*., 2021). Satya *et al*. (2017) reported that phosphate solubilizing bacteria (PSB) are ubiquitous. In other words, they are present in different types of soil. It was further reported that the population PSB in the soil is dependent depends on the chemical and physical properties of the soil, as well as the organic matter and phosphorus contents of the soil. The breaking down of insoluble phosphate into soluble form is carried out by some microbes present in the soil (Prajapati and Modi, 2012). Microorganisms with phosphate solubilizing potential increased the availability of soluble phosphate and enhanced the plant growth by improving biological nitrogen fixation.

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The fungi screened in this study did not fix nitrogen (Table 4). This finding is in line with the reports of Duggar and Davis (1916), who reported that nitrogen fixation could not be demonstrated by *Aspergillus niger, Macrosporium commune, Penictllium digitatum, P. expansum*, and *Glomerella gossypii*. The problem of fixation of free (atmospheric) or molecular nitrogen by the fungi has received attention by many researchers, yet a careful study of the literature is sufficient to indicate that much further work -with the strictest regards for accurate methods-will be required before the problem is satisfactorily solved. It is generally reported that fungi like *Pleurotus spp*. can fix nitrogen (N_2) (Jayasinghearachchi and Seneviratne 2004). The way they do it is still not clear, however, Jayasinghearachchi and Seneviratne (2004) hypothesized that only associations of fungi and diazotrophs can fix N_2.

A*spergillus niger* (OP970215) and *Penicillium chrysogenum* (OP970216) screened and identified in the study (Table 4)were among the profound efficient fungal species used for biofertilizer production. They have been known for solubilization of minerals in the soil (David *et al.,* 2023). Among the fungi tested, *Penicillium* sp, and *Aspergillus* sp. solubilized insoluble tricalcium phosphate. The phosphate-solubilizing ability of different isolates of fungi associated with legume root nodules under *invitro* conditions was evaluated by Caravaca *et al.* (2005) as a rock phosphate solubilizer. Kang *et al.* (2008) reported that a significant amount of insoluble rock phosphate was solubilized by *Aspergillus*sp. Which was evidenced by increased soluble phosphorus concentration. Singh *et al*. (2011) also tested and proved the ability of two *A. niger* strains as good phosphate solubilizers. Prajapati and Modi (2012), characterized both *A.niger* and *A. terreus* as potassium solubilizing fungi and the inoculation of the isolates with soil treated with insoluble potassium showed a significant increase in the concentration of potassium. Several studies have described the ability of *Aspergillus* sp.to solubilize phosphate and potassium in the soil (David *et al.,* 2023). Narshian and Patel (2000) used *A. aculeatus* isolates to test the availability of a named fungi in solubilizing rock phosphate and the researchers reported that *A. aculeatus* was capable of solubilizing all natural forms of phosphorus tested. The study of mineralization of organic phosphates and other minerals by *Aspergillus niger* and *Penicillium chrysogenum* as reported by Narshian and Patel (2002) and David *et al.* (2023), according to their reports, high acid phosphatase activity in Pikovskaya's broth was an indication that high percentage of phosphorus was being released. Therefore, microorganisms with phosphate solubilizing potential increased the availability of soluble phosphate and enhanced plant growth by improving biological nitrogen fixation.

Penicillium chrysogenum, P. pinophilum and few other species were evaluated by Fan *et al.* (2008) and David*et al.* (2023) in their abilities to improve the growth of plant. *P. pinophilum* formed *Arbuscular mycorrhizae* with the roots of strawberry and the interaction not only improved plant growth but also enhanced nutrient uptake as well as the rate of photosynthesis of the plant. *Penicillium chrysogenum* has been used to improve the growth of rice. The inoculum increased the nitrogen and phosphorus content as well as shortened the blossom and ripening period of strawberry (Aziz and Zainol, 2018).

CONCLUSION

In conclusion, it is observed in this study that microorganisms isolated from the farmland soil of University of Port Harcourt have biofertilizer potentials to improve plant growth by fixing nitrogen and solubilizing potassium and phosphate. It is proven that biofertilizers are capable of improving plant growth and soil fertility, therefore these microbes could be used to develop cost-effective and eco-friendly products that will serve as viable alternatives to chemical fertilizers for use as farm inputs to enhance plant growth, yield and disease control.

Conflict of Interest

The authors declare that there is no conflict of interest reported in this work.

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