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ISOLATION AND SCREENING FOR ANTIBIOTIC PRODUCTION FROM SOIL ACTINOMYCETES

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ABSTRACT

Actinomycetes are a group of gram-positive, filamentous, bacteria that are found mainly in soil, fresh water and decaying organic matter where they play an important role in decomposition, antibiotic production and nutrient cycle. The study was conducted to isolate, identify and screen the potential antimicrobial – producing *Actinomycetes* from the soil samples of Kano State Polytechnic Staff Quarters in Gwale Local Government area of Kano State. Three soil samples each were collected from five different sites. The physicochemical parameters of the soil samples showed variation according to the sampling sites. The soil type was found to be sandy, loamy and clay with brown and brownish black colors depending on the site. The soil pH was found to be at neutral to alkaline (7.33 ± 0.20 to 8.23 ± 0.25) and the temperature ranged from 28.33 ±1.52 to 31.66 ± 1.52. Electrical conductivity was recorded within the range of 1815.66 ± 83.51 to 9725.00 ± 16.12 µS/cm. All factors are within the normal ranges which support the growth of microorganisms. Using crowded plate technique, two species of *Actinomycetes* with antimicrobial potential were isolated from 15 soil samples at different sites around the rhizosphere of plants. The isolates were identified on the basis of colonial morphology and biochemical tests as *Streptomyces sp* and *Micromonospora sp* with frequency of occurrence of 60% and 40% respectively. Antibacterial activity of the *Actinomycetes* metabolites was tested against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeurginosa* using the disc diffusion assay. *Streptomyces* showed the highest antibacterial activity of 15.50±0.50 against *E. coli* and the lowest activity of 8.60±0.50 against *Pseudomonas aeruginosa*. *Micromonospora* was found to be less effective generally with an activity of 12.50±0.87 against *E.coli* and the lowest being 7.76±0.49 against *P. aeruginosa*. In conclusion, the findings from this study can be used for further investigation towards obtaining broad –spectrum antibiotics from soil microorganisms for therapeutic and industrial purposes.

Key words: Isolation, Soil Bacteria, Screening, Antibiotic, *Actinomycetes*, Kano State

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INTRODUCTION

Soil is a complex and diverse environment harboring different species of antibiotic producing organisms. Natural soil has a high biodiversity of bacteria and provides an important resource for possible antibiotics (Amin *et al*., 2015). Numerous factors including soil type, temperature, pH, salt concentration, carbon source, oxygen content, pressure, and others affect the microbial population in soil and the antibiotics diversities (Mannan*et al*., 2017). Most of the antibiotics are produced by soil microorganisms and natural soil provides the best medium for the growth of antibiotics producing microorganisms (Abbas *et al*., 2014). There are many microorganisms such as fungi, bacteria, *Actinomycetes*, which are potent antibiotics- producers that are widely used in today's scenario (Chandra and Kumar, 2017).

Actinomycetes are wide spread in soil and are considered as the most invaluable prokaryotes in medicinal and biotechnology industries because of their ability to produce number of bioactive molecules, particularly of the antibiotic compounds. *Streptomyces* that belong to the genus *Actinomycetes* has been considered for the production of 60% of the antibiotics (Kaur *et al*., 2014). It has been estimated that roughly two-third of the thousands of naturally occurring antibiotics have been isolated from *Actinomycetes* (Anuar *et al*., 2017). *Actinomycetes* are a prolific source of structurally diverse secondary metabolites; many of these possess pharmaceutically relevant biological activities (Al-Turk *et al*., 2020). Because of this, many scientists have focused on screening programs of microorganisms, primarily of *Actinomycetes*, for their production of antibiotics (Kakoi *et al*., 2016). The variety of terrestrial *Actinomycetes* are important in several areas of medicine and science for the production of antibiotics. Over the ancient decades, the bioactive compounds of these microorganisms have been utilized against number of bacterial infections. But as pathogens showed resistance to those previously available antibiotics, multi drug resistance turns out to be the most important problem of these days that is faced by everyone. So, there is a great need to discover novel antibiotics or to find out some other alternatives to fight against these pathogenic bacteria.

MATERIALS AND METHODS

STUDY SITES

The study was conducted at Kano State Polytechnic Staff Quarters along Sheik Jafar Road, Gwale Local Government area, Kano, Kano State.

Sampling Sites

The research was conducted on the soils of a cultivated land. The sampling sites were randomly selected around the rhizosphere of plants which is assumed to be inhabited by an active community of microorganisms. Five sampling sites were selected: Site A, Site B, Site C, Site D, and Site E.

Soil Sampling

A total of 15 samples were collected from the different sites by systematic random sampling with a total of three samples each from a site. The debris from the soil surface of the sampling sites were first removed before commencement of sampling. The samples were collected from the sites at the depth of 4-10 inches using a hand trowel sterilized with 70% ethanol and placed in sterile polythene bags. The samples were labeled as A1, A2, and A3,

B1, B2, and B3, C1, C2, and C3, D1, D2, and D3, E1, E2, and E3 and immediately transported to the laboratory for further analysis.

Determination of the Physicochemical Parameters of the Soil Samples

The Temperature, pH, and Electrical conductivity of the soil samples were determined according to the procedure describe by APHA (2005).

Serial Dilution of Soil Samples

One gram of soil was weighed and mixed in 10ml of sterile distilled water to get 1:10 dilution, then thoroughly mixed by vigorous shaking. This is taken as the stock solution. After allowing the sediment to settle, the supernatant was used for subsequent dilutions. Dilutions were prepared subsequently until 1:100000 dilutions are achieved.

Isolation of Soil *Actinomycetes*

A volume of 1 ml of suspension from 10^{-4} and 10^{-5} serially diluted tubes was spread evenly with sterile L-shaped glass rod over the surface of sterile starch casein agar aseptically using spread plating technique. Amoxicillin (20 µg/mL) and cyclohexamide (25 µg/ mL) was added to inhibit bacterial and fungal contamination, respectively. The plates were incubated aerobically at 37 °C for up to 7 days and observed intermittently during incubation. *Actinomycetes* on the plates were identified based on colonial morphology, color of hyphae and the presence or absence of aerial and substrate mycelium. Selected and identified colonies of *Actinomycetes* were transferred from the plate to starch casein agar slant and incubated at 37 °C for growth.

Physiological Characterization of the Isolated *Actinomycetes*

Individual colonies were characterized based on their color, shape, appearance, transparency, and Gram staining reaction.

Biochemical Screening of the Isolated *Actinomycetes*

Actinomycetes isolates were characterized biochemically to evaluate their chemical nature and identification by the following tests (Indole Production Test, Methyl Red Test, Voges-Proskauer Test, Citrate Utilization Test, Urease Test, Nitrate Reduction Test, Oxidase Test, Catalase Test, Gelatin Hydrolysis Test, Starch Hydrolysis, Triple Sugar Iron (TSI) Test as adopted after Olutiola*et al*., (2000) and Cheesebrough (2006).

Screening for Antibacterial Activity

Actinomycetes isolated and identified from different soil samples were screened for their antibacterial activity using Muller Hinton agar against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia.* Each plate was streaked with each of the test organisms. The overnight cultures of the colonies isolated were centrifuged and the supernatant was absorbed on paper discs. These discs were placed on the plates and incubated overnight and the zones of inhibition were measured and recorded.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was used to determine the effect of varying bacterial isolates. Least Significant Difference (LSD) was used to separate the means. All the analyses were carried out using SPSS software (20.0 version).

RESULTS AND DISCUSSION

The psychochemical parameters of the soil samples showed variation according to the sampling sites as shown in Table 1. The soil types at Site A and B were found to be loamy with brown to dark brown color, while Site C and E were found to be sandy and brownish in color. The soil type at Site D was clay with a brownish black color. The pH of the soil was found to be at neutral to slightly alkaline ranging from 7.33± 0.20 at Site C to 8.23± 0.25 at Site B. The temperature ranges from 28.33± 1.52 °C at Site B and D to 31.66 ±1.52 °C at Site C.

Table 1: Mean Values of Soil Physicochemical Parameters

Sample	Color of Soil	Type of Soil	pH (Mean ± SD)	Temperature (°C) (Mean ± SD)	EC (µS/cm) (Mean ± SD)
A	Dark brown	Loamy	7.80 ± 0.20^{b}	30.00 ± 1.00^{ab}	7455.00 ± 14.34^{b}
B	Brown	Loamy	8.23 ± 0.25^{c}	28.33 ± 1.52^{a}	7314.33 ± 24.32^{b}
C	Light brown	Sandy	7.33 ± 0.20^{a}	31.66 ± 1.52^{ab}	1815.66 ± 83.51^{a}
D	Brownish black	Clay	8.03 ± 0.15^{bc}	28.33 ± 1.52^{a}	9725.00 ± 16.12^{ab}
E	Brown	Sandy	7.86 ± 0.25^{bc}	29.66 ± 0.57^{ab}	1839.33 ± 22.94^{a}

Note: Values are mean ± SD. Mean with different superscript alphabet across the column are statistically significant ($p<0.05$)

Table 2 shows two species of *Actinomycetes* with potential antibiotic activity isolated from the soil, including *Streptomyces sp*. and *Micromonospora sp*. which were identified by colony morphology. *Streptomyces sp*. had large filamentous colonies, yellowish substrate mycelium and white aerial mycelium while *Micromonospora* sp. were short filamentous colonies, orange substrate mycelium, and had brownish aerial mycelium.

Table 2: Morphological Identification of *Actinomycetes* isolates

Isolate	Colony morphology	Substrate mycelium	Aerial mycelium
Streptomyces sp	Large filamentous Colonies	Yellowish	White
Micromonospora sp	Short filamentous Colonies	Orange	Brownish

Biochemical profiles revealed that *Streptomyces* was positive for nitrate reduction, starch hydrolysis, and TSI, but negative for indole, while *Micromonospora* was positive for indole and negative for nitrate reduction and starch hydrolysis. Both species were positive for citrate utilization, urease, oxidase, catalase, and gelatin hydrolysis (Table 3). This indicates that isolates confirm the metabolic versatility of *Actinomycetes* and their potential for producing diverse enzymes and antibiotics.

Table 3: Biochemical Identification of *Actinomycetes* isolates

Biochemical test	*Streptomyces* sp	*Micromonospora* sp
Indole	-	+
Methyl Red	-	-
Voges Proskeur	-	-
Citrate	+	+
Urease	+	+
Nitrate reduction	+	-
Oxidase	+	+
Catalase	+	+
Gelatin hydrolysis	+	+
Starch hydrolysis	+	-
Triple Sugar Iron	+	-

Key: Ind- Indole, MR- Methyl Red, VP- Voges Proskeur, Cit- citrate, Urea- Urease, Nit- Nitrate reduction, Oxid- Oxidase, Cat- Catalase, Gel- Gelatin hydrolysis, Starch- Starch hydrolysis, TSI- Triple Sugar Iron.

Figure 1 revealed the frequency of occurrence of the *Actinomycetes* in the soil samples. It shows *Streptomyces* to have the highest percentage of 60 and *Micromonospora* with a lower percentage of 40. The figure suggests that *Actinomycetes* distribution varied across soil samples with higher abundance in organic-rich, well-aerated soils (e.g., loamy soils), which aligns with the physicochemical results.

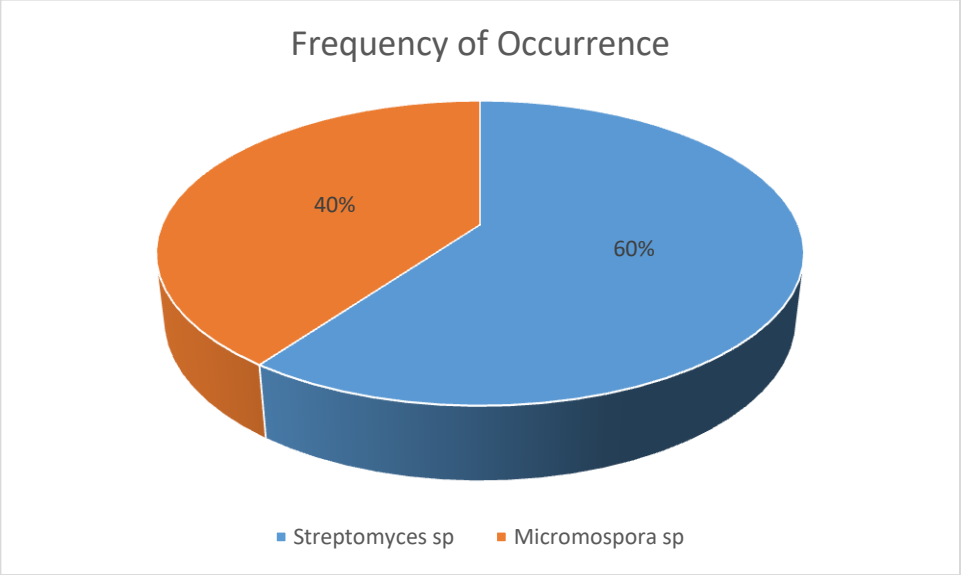


Figure 1: Frequency of Occurrence of *Actinomycetes* in the Soil Samples

Results of the antibacterial activity of *Actinomycetes* metabolites against the test organisms were presented in Table 4. *Streptomyces* showed the highest inhibition against *Escherichia coli* and the least against *Pseudomonas aeruginosa*. Metabolites from *Micromonospora* were found to be least effective with zones of inhibition ranging from lowest against *P. aeruginosa*to highest against *E. coli*. Both *Actinomycetes* isolates showed antibacterial

activity against all tested pathogens (*E. coli, S. aureus, K. pneumoniae, P. aeruginosa*), though less potent than the control antibiotic ciprofloxacin. *Streptomyces sp*. was the most effective against *E. coli* (15.50± 0.50 mm) while *Micromonospora sp*. was least effective against *P. aeruginosa* (7.76±0.49 mm). Ciprofloxacin showed significantly larger zones (17.82–22.65 mm), as expected for a broad-spectrum antibiotic. *Actinomycetes* metabolites have notable, though moderate, antibacterial properties. Their activity spectrum suggests potential for developing narrow-spectrum or synergistic antimicrobial agents.

Table 4: Antibacterial Activity of *Actinomycetes* metabolites against test organisms

	*Actinomycetes*Isolates	zones of inhibition (mm/d)			
		E.coli	*S. aureus*	*K. pneumoinae*	*P. aeruginosa*
1	*Streptomyces sp.*	15.50±0.50^{a}	10.73±0.64^{b}	10.76±0.25^{b}	8.60±0.50^{ab}
2	*Micromonospora* sp.	12.50±0.87^{ab}	11.23±0.30^{a}	9.63±0.41^{ab}	7.76±0.49^{a}
3	ciprofloxacin	22.54±0.91^{b}	22.65±1.25^{ab}	20.04±0.91^{a}	17.82±0.45^{b}

DISCUSSION

The mean ± standard deviation of the physicochemical parameters indicated that all sampling sites had alkaline soil pH (7.33 ± 0.20 to 8.23 ± 0.25), a range favorable for the growth of many bacteria. *Actinomycetes* and bacteria thrive in such pH levels due to optimal enzymatic and metabolic processes. These observations agree with a study by Akond *et al*., 2016 who reported a Ph range of 6.0 to 8.0 for the growth of *Streptomyces* and *Norcadia*. *Actinomycetes* grow best at 25–30°C, pH 6–8, and low moisture, although some species tolerate harsh conditions (Farda *et al*., 2022). However, the optimal pH for microbial growth may vary depending on nutrient availability. The soil types at the sampling sites were found to be loamy, sandy and clay in nature and around the rhizosphere of plants which was assumed to be rich in nutrients. Soil temperatures recorded across the sampling sites ranged between 28°C and 32°C, which supports the growth of mesophilic microorganisms such as the *Streptomyces* and *Micromonospora*. Microbial growth is directly dependent on temperature as a high temperature is known to denature their protein structure and a low temperature may inactivate enzymes and affects cellular metabolism. A research by Akond *et al*. (2016) reported a growth maximum temperature of 30^{o}C and 35^{o}C respectively for *Streptomyces* and *Norcadia*. The electrical conductivity (EC) which indicates the amount of soluble salts present in the soil also recorded variations during the research. The sandy soils at site C and E showed lower readings of 1815.66±83.51 and 1839.33±22.94 respectively which may be because of their larger particles and low water retention. Site A and B were loamy with EC of 7455.00±14.34 and 7314.33±24.32 indicating moderate salinity of the area. Site D with a clay type soil recorded the highest EC of 9725.00±16.12 indicating strong salinity and poor water drainage which may not support the growth of many microorganisms.

The antibacterial activity of the isolated *Actinomycetes* tested against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed significant difference with the highest inhibition zones against *E. coli* and the lowest against

Pseudomonas aeruginosa. The low inhibitory activity against *Pseudomonas aeruginosa* may be attributed to its extensive antimicrobial resistance due to intrinsic and acquired mechanisms including biofilm formation, multidrug efflux pumps and rapid acquisition of resistance genes.

Overall, Streptomyces exhibited stronger antimicrobial activity which may be attributed to its role as a major producer of two-thirds of all clinically useful natural antibiotics such as streptomycin, tetracycline, and neomycin, while *Micromonospora* produces fewer but important antibiotics such as gentamicin and related compounds. Morphological and physiological differences and similarities between the tested bacteria may be a determining factor in antibiotic activity and may account for differences in the zones of inhibition. Many researchers have also reported the antibacterial activity of *Actinomycetes* species against bacterial isolates (Dewi *et al*., 2025; Gitari *et al*., 2023; Sapkota *et al*., 2020; Kesavan and Selvam, 2013; Yücel and Yamaç, 2010; Al-Ajlani and Hasnain, 2010). The study reinforces the importance of microbial biodiversity in soil ecosystems and underscores *Actinomycetes* as a continuing source of bioactive compounds. With systematic exploration and modern biotechnological approaches, these organisms may contribute meaningfully to future therapeutic and industrial innovations.

Conclusion

This study successfully isolated and characterized *Actinomycetes* from diverse soil environments and evaluated their antibacterial potential. The findings confirm that soil physicochemical properties—particularly texture, pH, and salinity—influence the distribution and biological activity of *Actinomycetes*. Two morphologically and biochemically distinct isolates, *Streptomyces sp*. and *Micromonospora sp*., were identified, both of which demonstrated significant antimicrobial activity against a range of pathogenic bacteria, including *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa*. While the antibacterial effects of these isolates were less potent than the synthetic antibiotic ciprofloxacin, their broad-spectrum activity highlights the continued relevance of *Actinomycetes* as a valuable natural resource for antimicrobial discovery. This work supports the premise that soil microbes, especially in ecologically distinct regions, remain promising candidates in the search for novel bioactive compounds amid growing antibiotic resistance.

Recommendations

Based on the findings, the following recommendations are derived

1. Future studies should include a wider range of soil types (e.g., acidic, saline, or contaminated soils) and geographic locations to uncover novel *Actinomycetes* strains with unique metabolic and antimicrobial profiles.
2. The active compounds responsible for the observed antibacterial activity should be extracted, purified, and structurally identified using chromatographic and spectroscopic methods (e.g., HPLC, NMR, MS).
3. Isolates should be tested against multidrug-resistant (MDR) and clinically relevant strains to assess their potential in addressing current public health challenges

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