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Isolation and Characterization of Antibiotic-Producing Soil Microorganisms from Selected Agricultural Soils in Oko Community, Anambra State

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ABSTRACT

This study investigated the isolation and characterization of antibiotic-producing microorganisms from selected agricultural soils in Oko Community, Anambra State. Ten agricultural soil samples were collected and processed using standard microbiological techniques. The isolates were characterized on cultural and biochemical properties, and their antimicrobial activities were evaluated at 12.5, 25, 50, 75 and 100 mg/mL by the agar well diffusion method. Thirty bacterial isolates were recovered and presumptively identified as *Bacillus* sp. (36.7%), *Pseudomonas* spp. (26.7%), *Streptomyces* sp. (23.3%) and *Staphylococcus* sp. (13.3%). Antimicrobial screening showed concentration-dependent inhibition. *Streptomyces* sp. exhibited the highest activity, with an inhibition zone of 28.9 ± 0.5 mm at 100 mg/mL, followed by *Bacillus* sp. (26.8 ± 0.4 mm), *Staphylococcus* sp. (25.6 ± 0.3 mm) and *Pseudomonas* spp. (22.7 ± 0.4 mm). No inhibition was observed with the DMSO negative control. Agricultural soils in Oko Community therefore harbour cultivable microorganisms with promising antimicrobial activity, particularly *Streptomyces* and *Bacillus*. Further molecular identification and chemical characterization of the active metabolites are recommended.

Keywords: agricultural soil; antimicrobial activity; antibiotic-producing microorganisms; *Bacillus*; *Streptomyces*; Oko Community

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1. Introduction

Antibiotics remain among the most important products obtained from microorganisms. Their discovery transformed the management of infectious diseases, but the usefulness of many existing agents is threatened by antimicrobial resistance. Bacteria can develop resistance by modifying drug targets, enzymatically inactivating antimicrobial agents, reducing permeability, and actively effluxing drugs. The spread of resistant microorganisms has created a continuing need for new antimicrobial compounds and alternative sources of bioactive metabolites [13,17].

Soil is one of the most biologically active natural environments and contains an enormous diversity of microorganisms. Bacteria, fungi, actinomycetes, archaea and protozoa coexist and interact through competition, antagonism, nutrient cycling and symbiosis. These interactions may stimulate production of secondary metabolites with antibacterial, antifungal, antiparasitic, anticancer or immunosuppressive activity. Among soil microorganisms, actinomycetes have received particular attention. Members of *Streptomyces* have contributed substantially to the discovery of clinically useful antibiotics. They are filamentous Gram-positive bacteria involved in the decomposition of complex organic materials, and their metabolic versatility enables them to produce compounds that inhibit

competitors. Less frequently studied environments may still contain strains capable of producing previously uncharacterized bioactive compounds [3,8].

Murray et al. reported that bacterial antimicrobial resistance was associated with millions of deaths globally in 2019 [13]. Agricultural soils are a relevant setting for this search. They are influenced by plant roots, decaying organic matter, nutrient availability, moisture, crop type and agricultural practice, and they support complex microbial communities. Production of inhibitory metabolites may be one mechanism by which certain microorganisms establish themselves in a competitive environment [3]. Changes in pH, organic matter, nutrients, moisture and vegetation can alter community composition and activity, so isolation from different agricultural soils may recover organisms with different antimicrobial capabilities [16].

Oko Community, in Orumba North Local Government Area of Anambra State, has agricultural land that may harbour diverse soil microorganisms. The antibiotic-producing potential of cultivable organisms from selected soils in this community has not been systematically reported. Local screening can add to knowledge of microbial diversity while identifying isolates that inhibit selected test organisms.

Discovery of antibiotic-producing microorganisms generally proceeds by aseptic collection, isolation on appropriate media, purification, cultural and biochemical characterization, and preliminary screening for inhibition. Isolates with reproducible activity may then be taken forward for molecular identification and chemical investigation of the active compounds [2,10]. An inhibition zone indicates antimicrobial activity under the conditions of the assay. It does not, by itself, establish that a chemically identified antibiotic has been produced. Isolation, purification and characterization of the active compound are required before a metabolite can be described as a novel antibiotic [2,8].

This study examined ten agricultural soil samples from Oko Community in order to isolate and characterize cultivable soil microorganisms and to screen them for antibiotic-producing potential.

2. Materials and methods

2.1 Study area and samples

Agricultural soil was collected from selected farming locations in Oko Community, Orumba North Local Government Area, Anambra State, Nigeria. Sampling locations were documented, and geographical coordinates were recorded where a GPS device was available. Ten samples, coded S1 to S10, were processed separately so that organisms recovered from each location could be compared.

2.2 Collection

Surface leaves, stones and plant debris were removed. Approximately 10 g of soil was collected aseptically from 5–10 cm below the surface with a sterile auger or spatula and transferred into a labelled sterile container. Samples were transported to the microbiology laboratory and processed as soon as possible.

2.3 Culture media

Nutrient agar was used for isolation and maintenance of general bacterial isolates. Starch casein agar was used for recovery of actinomycetes, particularly suspected *Streptomyces*. Mueller–Hinton agar was used for the antimicrobial assay. Media were prepared according to the manufacturer's instructions and sterilized before use. Standardized testing conditions were used because medium composition can influence antimicrobial results [6].

2.4 Suspension, dilution and isolation

One gram of each soil sample was suspended in 9 mL of sterile physiological saline (10^{-1}). Ten-fold serial dilutions were prepared to 10^{-6} where necessary. Selected dilutions (0.1 mL) were spread on the appropriate agar. Plates with discrete colonies were examined, and colonies differing in colour, shape, elevation, margin and surface were subcultured until pure cultures were obtained [4].

2.5 Isolation of actinomycetes

Selected dilutions were inoculated onto starch casein agar and incubated for an extended period. Colonies with dry, powdery, leathery or filamentous growth, and in some cases aerial mycelia or pigment, were selected, purified and characterized. Actinomycetes were included because *Streptomyces* is a recognized producer of antimicrobial secondary metabolites [3,8].

2.6 Purification and preservation

Distinct colonies were streaked onto fresh agar until colony morphology was consistent. Purity was assessed by colony characteristics and microscopy. Isolates were coded by sample (for example S1A, S1B, S1C) and maintained on agar slants.

2.7 Cultural and biochemical characterization

Colony colour, shape, margin, elevation and surface were recorded. Biochemical tests were Gram staining, catalase, oxidase, citrate utilization, urease and starch hydrolysis. Results were interpreted together with cultural characteristics for presumptive identification as *Bacillus* sp., *Streptomyces* sp., *Staphylococcus* sp. or *Pseudomonas* spp.

2.8 Antimicrobial screening

Preparations from the suspected antibiotic-producing isolates were tested at 12.5, 25, 50, 75 and 100 mg/mL by agar well diffusion. A standardized suspension of each test organism was spread on agar, wells of uniform diameter were cut, and the preparations were added. Ciprofloxacin was the positive control and dimethyl sulphoxide (DMSO) the negative control. After incubation, inhibition zones were measured in millimetres and expressed as mean $\pm$ standard deviation. Zone diameter was interpreted as evidence of activity, not as a direct measure of potency, because diffusion, molecular size and solubility also affect zone size [2].

2.9 Minimum inhibitory concentration

The manuscript reports a minimum inhibitory concentration (MIC) series at the same concentrations (12.5–100 mg/mL), defined as the lowest concentration preventing visible growth, with growth and sterility controls, following the principle of dilution susceptibility testing [6]. The tabulated values supplied with the manuscript are inhibition-zone measurements rather than growth/no-growth dilution results; that distinction is retained in the discussion.

3. Results

Thirty bacterial isolates were recovered from the ten soil samples and presumptively identified as *Bacillus* sp. (11/30, 36.7%), *Pseudomonas* spp. (8/30, 26.7%), *Streptomyces* sp. (7/30, 23.3%) and *Staphylococcus* sp. (4/30, 13.3%). Cultural characteristics are given in Table 1 and biochemical reactions in Table 2.

Table 1. Cultural characteristics of microorganisms isolated from agricultural soils in Oko Community

Sample	Organism	Colour	Shape	Margin	Elevation	Surface
S1A	<i>Bacillus</i> sp.	Cream	Circular	Entire	Convex	Smooth
S1B	<i>Streptomyces</i> sp.	White	Irregular	Undulate	Raised	Dry
S1C	<i>Staphylococcus</i> sp.	Yellow	Circular	Entire	Convex	Smooth
S2A	<i>Bacillus</i> sp.	Cream	Circular	Entire	Convex	Smooth
S2B	<i>Pseudomonas</i> spp.	Whitish-grey	Irregular	Filamentous	Raised	Dry/powdery
S2C	<i>Bacillus</i> sp.	Cream	Circular	Entire	Convex	Smooth
S3A	<i>Streptomyces</i> sp.	White	Circular	Entire	Convex	Smooth
S3B	<i>Bacillus</i> sp.	Cream	Irregular	Undulate	Raised	Rough
S3C	<i>Pseudomonas</i> spp.	Grey	Irregular	Filamentous	Flat	Dry/powdery
S3D	<i>Staphylococcus</i> sp.	Yellowish	Circular	Entire	Convex	Smooth
S4A	<i>Bacillus</i> sp.	Cream	Circular	Entire	Convex	Smooth
S4B	<i>Streptomyces</i> sp.	White	Irregular	Undulate	Raised	Dry
S5A	<i>Pseudomonas</i> spp.	Grey	Irregular	Filamentous	Raised	Powdery
S5B	<i>Bacillus</i> sp.	Cream	Circular	Entire	Convex	Smooth
S5C	<i>Staphylococcus</i> sp.	Yellow	Circular	Entire	Convex	Smooth
S6A	<i>Streptomyces</i> sp.	White	Circular	Entire	Convex	Smooth
S6B	<i>Pseudomonas</i> spp.	Greyish-white	Irregular	Filamentous	Raised	Dry
S6C	<i>Bacillus</i> sp.	Cream	Circular	Entire	Convex	Smooth

Sample	Organism	Colour	Shape	Margin	Elevation	Surface
S7A	<i>Bacillus</i> sp.	Cream	Circular	Entire	Convex	Smooth
S7B	<i>Streptomyces</i> sp.	White	Irregular	Undulate	Raised	Rough
S7C	<i>Pseudomonas</i> spp.	Grey	Filamentous	Filamentous	Raised	Powdery
S8A	<i>Staphylococcus</i> sp.	Yellow	Circular	Entire	Convex	Smooth
S8B	<i>Bacillus</i> sp.	Cream	Circular	Entire	Convex	Smooth
S8C	<i>Streptomyces</i> sp.	White	Irregular	Undulate	Raised	Dry
S9A	<i>Pseudomonas</i> spp.	Grey	Irregular	Filamentous	Flat	Powdery
S9B	<i>Bacillus</i> sp.	Cream	Circular	Entire	Convex	Smooth
S9C	<i>Streptomyces</i> sp.	White	Irregular	Undulate	Raised	Rough
S10A	<i>Bacillus</i> sp.	Cream	Circular	Entire	Convex	Smooth
S10B	<i>Pseudomonas</i> spp.	Whitish-grey	Irregular	Filamentous	Raised	Dry
S10C	<i>Staphylococcus</i> sp.	Yellow	Circular	Entire	Convex	Smooth

Table 2. Biochemical characteristics of selected soil isolates

Isolate	Gram	Catalase	Oxidase	Citrate	Urease	Starch
<i>Bacillus</i> sp.	+	+	–	+	–	+
<i>Streptomyces</i> sp.	+	+	–	+	–	+
<i>Staphylococcus</i> sp.	+	+	–	–	–	–
<i>Pseudomonas</i> spp.	–	+	+	+	–	+

Key: + = positive; – = negative.

Antimicrobial screening showed concentration-dependent inhibition. At 100 mg/mL, *Streptomyces* sp. produced the largest zone (28.9 ± 0.5 mm), followed by *Bacillus* sp. (26.8 ± 0.4 mm), *Staphylococcus* sp. (25.6 ± 0.3 mm) and *Pseudomonas* spp. (22.7 ± 0.4 mm). DMSO produced no zone. The source table headed these columns as a ciprofloxacin positive control, but no separate ciprofloxacin zone diameters were supplied; the values below are those reported for the isolate preparations and match the abstract.

Table 3. Antimicrobial activity of suspected antibiotic-producing soil isolates (inhibition zone, mm; mean $\pm$ SD)

Organism	12.5 mg/mL	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	DMSO
<i>Bacillus</i> sp.	10.8 ± 0.4	14.6 ± 0.3	18.7 ± 0.5	22.6 ± 0.6	26.8 ± 0.4	0.0 ± 0.0
<i>Streptomyces</i> sp.	11.5 ± 0.5	16.2 ± 0.4	20.1 ± 0.6	24.3 ± 0.5	28.9 ± 0.5	0.0 ± 0.0
<i>Staphylococcus</i> sp.	9.6 ± 0.3	13.8 ± 0.5	17.4 ± 0.4	21.5 ± 0.4	25.6 ± 0.3	0.0 ± 0.0
<i>Pseudomonas</i> spp.	8.7 ± 0.4	12.3 ± 0.4	15.9 ± 0.5	19.6 ± 0.4	22.7 ± 0.4	0.0 ± 0.0

Table 4 repeats the same zone diameters and records 12.5 mg/mL as the MIC for every organism, because that was the lowest concentration tested and it produced a measurable zone. A dilution MIC was not separately tabulated.

Table 4. Zone diameters used by the author to assign an apparent MIC for the most active preparation

Organism	12.5 mg/mL	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	Reported MIC
<i>Bacillus</i> sp.	10.8 ± 0.4	14.6 ± 0.3	18.7 ± 0.5	22.6 ± 0.6	26.8 ± 0.4	12.5
<i>Streptomyces</i> sp.	11.5 ± 0.5	16.2 ± 0.4	20.1 ± 0.6	24.3 ± 0.5	28.9 ± 0.5	12.5
<i>Staphylococcus</i> sp.	9.6 ± 0.3	13.8 ± 0.5	17.4 ± 0.4	21.5 ± 0.4	25.6 ± 0.3	12.5
<i>Pseudomonas</i> spp.	8.7 ± 0.4	12.3 ± 0.4	15.9 ± 0.5	19.6 ± 0.4	22.7 ± 0.4	12.5

4. Discussion

Thirty isolates were recovered from ten agricultural soils and provisionally grouped by colonial morphology, Gram reaction and selected biochemical tests. *Bacillus* sp. was the most frequent (36.7%). Colonies were mostly cream, circular, convex, smooth and entire, with some irregular, undulate, raised or rough forms, consistent with the range described for *Bacillus* and with the ability of the genus to persist in soil by endospore formation [12]. Soil *Bacillus*

isolates are recognized sources of antibacterial metabolites, including bacteriocins, lipopeptides and polyketides [15,18].

Streptomyces sp. accounted for 23.3% of isolates. Most colonies were white, irregular or undulate, raised, and dry, rough or powdery, in keeping with aerial mycelium and sporulation in soil streptomycetes [1]. Recovery of this genus is relevant because a large share of known microbial antibiotics has come from *Streptomyces*, which carries extensive biosynthetic capacity for antibacterial, antifungal and other metabolites [1,8].

Staphylococcus sp. (13.3%) formed yellow or yellowish, circular, smooth, entire, convex colonies and was Gram-positive and catalase-positive, which distinguishes staphylococci from catalase-negative Gram-positive cocci [14]. *Pseudomonas* spp. (26.7%) were generally grey or whitish-grey, irregular, and dry or powdery, and were Gram-negative, catalase-positive, oxidase-positive and citrate-positive. Soil pseudomonads are also known to produce antimicrobial metabolites and to compete in the rhizosphere [9]. Together, the four groups show that these agricultural soils contain genera already associated with antimicrobial secondary metabolism.

Biochemical patterns supported the presumptive identities. *Bacillus* sp. and *Streptomyces* sp. were Gram-positive, catalase-positive, citrate-positive, urease-negative and starch-hydrolysis-positive. Starch hydrolysis in *Streptomyces* is consistent with extracellular enzymes used to degrade complex soil substrates [1]. *Staphylococcus* sp. was negative for oxidase, citrate, urease and starch hydrolysis. *Pseudomonas* spp. were oxidase-positive and citrate-positive. These profiles are preliminary. Species-level assignment would require molecular identification, such as 16S rRNA sequencing, which was not done here [11].

Inhibition increased with concentration for all four groups. *Streptomyces* sp. rose from 11.5 ± 0.5 mm at 12.5 mg/mL to 28.9 ± 0.5 mm at 100 mg/mL, in line with the genus as a major source of secondary metabolites [1]. *Bacillus* sp. rose from 10.8 ± 0.4 mm to 26.8 ± 0.4 mm. *Staphylococcus* sp. rose from 9.6 ± 0.3 mm to 25.6 ± 0.3 mm, and *Pseudomonas* spp. from 8.7 ± 0.4 mm to 22.7 ± 0.4 mm. The smaller zones against *Pseudomonas* spp. may reflect the Gram-negative outer membrane and efflux systems rather than absence of activity [5]. Zone diameter is also affected by diffusion, molecular size and solubility, so it is evidence of activity, not a direct measure of potency [2].

DMSO produced no zone, which supports attribution of the observed inhibition to the test preparation rather than the solvent. At 100 mg/mL the order was *Streptomyces* sp. > *Bacillus* sp. > *Staphylococcus* sp. > *Pseudomonas* spp. The manuscript also states that ciprofloxacin was included as a positive control, but zone diameters for that control were not reported, so the assay cannot be compared numerically with the reference antibiotic.

The lowest concentration tested, 12.5 mg/mL, produced a measurable zone against all four organisms, and Table 4 therefore records 12.5 mg/mL as the MIC. That figure is not a conventional MIC. A true MIC is the lowest concentration that prevents visible growth in a broth or agar dilution test [6]. Selecting the lowest concentration that produces a zone in an agar diffusion assay does not establish that endpoint. The 12.5 mg/mL value should be read as the lowest concentration tested that showed activity, not as a validated MIC.

5. Conclusion

Agricultural soils in Oko Community yielded 30 cultivable isolates, presumptively *Bacillus* sp., *Pseudomonas* spp., *Streptomyces* sp. and *Staphylococcus* sp., with *Bacillus* sp. predominant. The preparations showed concentration-dependent antimicrobial activity. *Streptomyces* sp. gave the largest zone, 28.9 ± 0.5 mm at 100 mg/mL. These soils are a useful source of microorganisms for further antibiotic screening. Molecular identification of the isolates, and isolation and chemical characterization of the active metabolites, are required before any compound can be described as an antibiotic.

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