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Qualitative and Quantitative Screening of Actinomycetes from Farmland and Rhizospheric Soil

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Abstract: Farmland soil and rhizospheric soil were screened for the presence of antibiotic producing Actinomycetes. Using culture based techniques. Isolates were isolated suaing two media: Starch Casein Agar medium and Actinomycetes Isolation Agar (AIA) medium. Isolates were characterized morphologically and biochemically and identified to be in the genus Actinomycetes. Ten isolates were isolated with varied qualitative and quantitative spectrum of activity against Multidrug resistant *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Candida albicans*. The quantitative antimicrobial assay recorded a zone of inhibition of 0-26mm diameter of zone of inhibition. Results show that the soil samples are important sources of antibiotics for the fight of Multidrug resistant isolates.

Keyword: Actinomycetes, Antibiotics, Multidrug Resistance, Rhizopheric Soil

Introduction

Continuous efforts have been put in place to isolate, characterize and identify antibiotic producing microorganisms in a bid to join the quest against multiple drug resistance [1,2]. Sources of Antibiotics are numerous and include animal, plants and microbial sources, but much interest have been given to microorganisms due to their ease of cultivation, fast growth rate and generation time and the ease of purification of metabolites [3]. Soil represents a rich diversity of microorganism and as a rich media, provides support for both beneficial and harmful microorganisms [4,2]. Similarly, Exudates from plants makes the rhizosphere a rich source of microbial diversity. Many competitive microorganisms are found in such environment that have the capacity to produce antimicrobial substances against other microbes. Berdy [3] explains that the products of biological origin that have antibiotic properties are usually produced as secondary metabolites which are usually in the idiophase-stationary phase in the bacterial growth curve. Hence, the need for prolonged incubation. Among other group of bacteria, interest in Actinomycetes is vast as a result of their availability in both natural and manmade environment; and their distribution is dependent on physico-chemical and environmental factors [5, 6]. Bioactive compounds produced by actinomycetes are vast and many have been characterized, purified and applied in the fight against microbial infections and diseases and records about 70% of all produced antibiotics of microbial origin [7,8]. Indeed their products have been used against fungi, bacteria, tumors and cancer, and some have been reported to have immune suppressive properties [3,9, 10, 11]. Hence, this work screens Actinomycetes isolated from a farmland and rhizospheric soil in order to determine their capacity to produce antimicrobial metabolites that will demonstrate activity against selected multidrug resistant isolates.

Materials and Methods

Soil sample collection

Ten (10) soil samples were collected from the rhizosphere of plants and agricultural soils from FUTO farms. Farmland soils were excavated from depth of 5 to 15 cm by using a soil Auger and collected in clean, dry and sterile polyethylene bags. The Rhizospeheric soils were collected as soils attached to the surface of plants' roots. All samples were appropriately labeled, transported to the Laboratory and stored in the refrigerator at 4°C for further investigations.

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Preparation of Media for the cultivation of Actinomycetes

Following the methods described by Awad *et al*. [12] and Arifuzzaman *et al*. [13] for isolation of Actinomycetes, two separate media were prepared: Starch Casein Agar medium (SCA, g/L) containing soluble starch 10; casein, 0.3; KNO_3 2, NaCl 2, K_2HP0_4 2, $MgSO_4.7H_2O$ 0.05, $CaCO_3$ 0.02, $FeSO_4.7H_2O$ 0.01; agar, 15; and the final pH was adjusted to 7.0±2 prior to sterilization [13]; and the Actinomycetes Isolation Agar (AIA) medium of g/L containing heart infusion broth, 25.0; casein hydrolysate, 4.0; yeast extract, 5.0; dextrose, 5.0; cysteine HCl, 1.0; soluble starch, 1.0; potassium phosphate, 15.0; ammonium phosphate, 1.0; magnesium sulphate, 0.2; calcium chloride, 0.02; Agar, 20; and adjusted to a neutral pH before sterilization. The two media were autoclaved at 121°C for 15 min; cooled to 50°C and supplemented with 50 µg/ml of amphotericin B to inhibit fungal growth. The media was plated onto sterile disposable petri dishes, allowed to solidify, surfaced dried and incubated overnight for sterility testing.

Isolation of Actinomycetes and pure culture technique

Actinomycetes were isolated using the spread plate technique following a tenfold serial dilution up to the 7^{th} diluent. The resulting suspensions (diluents) were agitated with a vortex and 0.1 ml of suspension was taken from 10^{-3}, 10^{-5}, and 10^{-7} diluents and spread on each of the surface dried and sterile Starch Casein Agar medium and Actinomycetes Isolation Agar medium and incubated at 30°C for 7 to 10 days [14]. Isolated colonies were characterized based on colonial morphology and colour of mycelium according to methods described by Williams and Cross [15]. Pure cultures were obtained using pure culture techniques and stored in agar slants at 4 ^{o}C.

Screening of antimicrobial activity of actinomycetes isolates against test microorganisms
Collection and Standardization of Test Isolates

The test organisms used for antimicrobial screening were all Multidrug Resistant clinical isolates obtained from the Federal Medical Centre, Oweri, Imo state, Nigeria. They include: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Candida albicans*. The identity of the isolates were identified using Biochemical methods described by Cheesbrough [16]. Each Isolate was standardized using MacFarland standard 0.5 Using methods described by Lalitha [17].

Primary screening

A total of 10 isolates were primarily screened for antimicrobial activity against five test microorganisms according to Methods described by Pandey *et al.* [18]. Seven day grown isolates were streaked as a straight line across diameter on Nutrient Agar plates (Oxoid) and incubated at 30°C for 6 days. After 6 days, Standardized test microorganisms, namely, *S. aureus*, *E. coli*, *P. aeruginosa* and *Salmonella typhi*, and *Candida albicans* from overnight culture broth were streaked at right angle, but not touching the streaked isolate and incubated at 37°C for 24 h. Clearing zone formation between the antibiotic producing isolate and the test organisms was considered positive for antibiotic production. The isolates were then selected based on a wide spectrum activity against tested microorganisms.

Secondary screening

Broth cultures with of the isolates were incubated at 30 degrees in Starch Caesin Broth for 9 days. The broth was filtered using a Whatman filter paper No 1 and the filtrate, precipitated with Ethyl acetate and separated according to methods described by Atsede and Fassil [19] was tested against the test isolates using the disc diffusion technique. These isolates were characterized using Biochemical characteristics including gelatin hydrolysis, starch hydrolysis, and esculin degradation as described by by Atsede and Fassil [19].

Analysis of the results

Results was subjected to statistical analysis using Microsoft Excel 2016 and the results of triplicate experiments were reported as Mean±SD

RESULTS

Table 1 shows the recovery rates of isolates from the Rhizospheric soil and farmland soil. Ten isolates were recovered with 6(60%) and 4(40%) obtained from Rhizospheric soil and Farmland Soil respectively. This implies that a greater recovery was observed in Rhizospheric soil than Farmland soil respectively. 70% of the isolates recovered were obtained from the Actinomycetes Isolation Agar medium while the Starch Casein Agar medium had a 40% recovery. In the cases of equal recovery, both isolates were tested for the assay and the positive sample was reported. The test isolates were confirmed for their identity using biochemical tests displayed in Table 3.

Isolates recovered from the Soil samples were screened for antibiotic production based on their antimicrobial properties against isolates and the results are displayed in table 2. Two isolates from the rhizospheric soil, X10R and X5R, had highest recorded antibacterial and antifungal activity recording an inhibition of 4 out of the 5 test isolates. On the other hand, X6S recorded the least spectrum of activity by inhibiting only *P. aeruginosa. S. aureus*, *P. aeruginosa* and *Salmonella typhi* were inhibited by 60% of the screened Actinomycetes isolates. Escherichia soli and Candida albicans recorded 40% and 20% inhibition by tested Actinomycetes isolates. Table 4 shows the

antimicrobial assay results using the disk diffusion technique most recorded antimicrobial activity recorded were within the range of 8-26mm diameter of zone of inhibition.

Table 1. Recovery of Actinomycetes from the different soil samples

Codes of isolates	Source of Sample	
	Rhizospheric soil (R)	Farmland Soil (S)
X1	-	-
X2	-	+
X3	+	_
X4	+	_
X5	+	+
X6	-	+
X7	+	+
X8	_	-
X9	+	-
X10	+	-
Total	6(60%)	4(40%)

Table 2. Qualitative Screening of antibiotic producing capabilities of the isolates (Antimicrobial Assay)

ISOLATE	TEST ISOLATES					SPECTRUM OF ACTIVITY
	S. aureus	*E. coli*	*P. aeruginosa*	*Salmonella typhi*	*Candida albicans*	
X2S	+	-	+	+	-	3
X3R	-	+	+	-	-	2
X4R	+	+	-	-	-	2
X5R	+	+	-	+	+	4
X5S	+	-	-	+	-	2
X6S	-	-	+	-	-	1
X7R	+	-	+	-	-	2
X7S	+	-	-	+	-	2
X9R	-	-	+	+	-	2
X10R	-	+	+	+	+	4
Total	6(60%)	4(40%)	6(60%)	6(60%)	2(20)	

+, Active against test organism; -, inactive against test organism. *Show broad spectrum activity.

Table 4. Quantitative Screening of antibiotic producing capabilities of the isolates (Antimicrobial Assay)

ISOLATE	TEST ISOLATES					SPECTRUM OF ACTIVITY
	S. aureus	*E. coli*	*P. aeruginosa*	*Salmonella typhi*	*Candida albicans*	
X2S	26±0.2	11±0.3	12±0.3	11±0.2	8±0.3	3
X3R	10±0.2	11±0.1	10±0.2	12±0.1	11±0.2	2
X4R	17±0.1	13±0.3	12±0.3	0	0	2
X5R	20±0.2	14±0.1	11±0.3	11±0.2	10±0.1	4
X5S	13±0.1	0	0	13±0.1	0	2
X6S	0	0	12±0.2	0	0	1
X7R	14±0.1	0	13±0.1	0	0	2
X7S	18±0.1	0	0	11±0.3	0	2
X9R	0	0	13±0.3	14±0.2	0	2
X10R	0	12±0.1	11±0.3	16±0.1	13±0.2	4

Table 3. Biochemical characterizaton of clinical isolates

Cat	Oxi	Coa	Ind	Met	Vog	Cit	Ure	NO_3	Glu	Suc	Lac	Mal	Man	GTT	H_2S	Identity of isolates
+	-	+	-	-	+	-	+	+	+	+	+	+	+	Nd	-	*Staphylococcus aureus*
+	+	-	-	+	-	+	+	+	+	-	-	-	+	Nd	+	*Pseudomonas aeruginosa*
+	-	-	+	+	-	-	-	+	+	+	+	+	+	Nd	-	*Escherichia coli*
-	-	-	-	+	-	+	-	-	+	+	+	-	+	Nd	-	*Staphylococcus aureus*
+	-	-	-	Nd	nd	nd	nd	nd		+	+	+	+	+	nd	*Candida albicans*

Cat=catalase, Cit= Citrate, Coa=coagulase, Glu=glucose, H_2S=Sulfide reduction, Ind=Indole, Lac= Lactose, Mal=Maltose, Man=Mannose, Met=Methyl Red, NO_3=Nitrate reduction, Oxi=oxidase, Suc= Sucrose, Ure=Urease, Vog=Voges Proskeur, GTT= Germ Tube Test

Discussion

As important as antibiotics are in Nature, so also are the defenses mounted against them constantly evolving. Abo-Shadi *et al*. [20] reported that due to the burden for high frequency of multidrug resistant pathogens in the world, there has been increasing interest for searching effective antibiotics from soil Actinomycetes in diversified ecological niches. Based on the biochemical characteristics obtained in this work for the isolated isolates, the isolates are classified under the genus Actinomycetes [21, 15].

Antimicrobial properties of the isolates observed qualitatively and quantitatively through the Observation of clear inhibition zones around the streaks and wells on the inoculated plates is an indication of antimicrobial activities of antibiotics produced and extracted from the Actinomycetes against test organisms. Similar results of 0-26mm diameter of zone of inhibition that was recorded in this workhave been reported by Gurung *et al*. [5]. However, results were considerably lower that results obtained from and Bizuye *et al*. [6] who recorded a 0-40mm diameter of zone of inhibition.

Conclusion and recommendation

As microbes are diverse, so are their metabolites. Antibiotics are indeed produced by the group of microorganisms known as the Actinomycetes and the need to explore these organisms for this product is vital in the fight against antibiotic resistance. Genetic and molecular tools will play vital roles in our subsequent research where we will explore the gene that is necessary for the production of the antibiotics, Purify and study the structures of the antibiotics, Mutate the gene for future yield and optimize physiological parameters for the optimum production of antibiotics.

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