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ANTIFUNGAL POTENTIAL OF ACTINOMYCETES ISOLATED FROM FIVE DIFFERENT SOURCES AGAINST *Aspergillus flavus* AND *Fusarium verticillioides*

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ABSTRACT
This research work is to investigate the antifungal potential of actinomycetes isolated from five different sources against *Aspergillus flavus* and *Fusarium verticillioides*. Isolation of microorganisms from the soil samples was carried out by serial dilution and pour plate techniques. The soil isolates were screened for their antagonistic ability against mycotoxigenic fungi: *A. flavus* and *F. verticillioides*. The isolates include *Actinomyces* spp, *Mycobacterium* spp, *Rhodococcus* spp, *Nocardia* spp, *Streptomyces* spp, and *Streptomonospora* spp. The results indicate that all the bacteria species exhibited varying degrees of biological control potential against the tested fungi. Out of all the actinomycetes use, *Streptomyces* spp appeared to have the highest diameter zone of inhibition against *A. flavus* of 16.00 mm and against *F. verticillioides* of 21.00 mm. The results showed the antimicrobial activity of the actinomycetes species, and thus indicate the potential of using them as bio-fungicidal agents in reducing the damages and threat caused by *A. flavus* and *F. verticillioides* in crop production.

1.0 Introduction

Microorganisms are ubiquitous that can occur in a wide diversity of taxonomic group which include bacteria, algae, fungi, protozoa and viruses. Microorganisms can be of beneficial and necessary for human well-being, and some of their microbial activities may cause undesirable consequences. Microorganisms found in soils are of the great important in fighting against the biological activity of a wide range of pathogens. Microbes produce bioactive molecules which are not used for their growth and development but useful in defence mechanism (Braga *et al.*, 2016).

Some research works have been carried out to control the pathogens and to identify new antimicrobial agents. Actinomycetes member of a heterogenous group of Gram-positive, anaerobic bacteria found in soil which is harmless to animals and higher plants but posses ability to produce a wide range of biologically active secondary metabolites, which are very effective against microbial pathogens (Rahman *et al.*, 2011). The genus *Streptomyces* is the biggest producer of antibiotics. Several

microbial secondary metabolites are reported to be rich sources of therapeutic drugs (Wohlleben *et al*., 2016).

Aspergillus flavus and *Fusarium verticillioides* are widespread inhabitants of agricultural soils associated with many crops. They cause economic losses through diseases they produce and are of a threat to human and animal health due to the contamination of crops with aflatoxins and fumonisins they produce (Egbuta *et al*., 2017). Effective control traditionally has been through the use of chemical fungicides, modifications in cultural practices, and development of resistant cultivars and methods of postharvest sorting of contaminated products to reduce mycotoxin content (Loi *et al*., 2017).

Therefore, the objective of the present study is to evaluate the antifungal potential of actinomycetes isolated from five different sources against *A. flavus* and *F. verticillioides* to reduce crops contamination and loss.

2.0 Materials and Method

2.1 Sources of soil samples

Soil samples were collected from Akungba-Akoko metropolis in five different sites which includes; maize plantation in Adekunle Ajasin University's (AAUA) permanent site, Orange plantation in Ilale area, Microbiology Laboratory area, Okusa area and Ibaka areausing well labelled sterile transparent polyethylene bagswhich was immediately transported to theMicrobiology laboratory, Adekunle Ajasin University Akungba-Akoko for further microbiological analyses.

2.2 Isolation of pure culture of *actinomycetes*

Different species of actinomycetes were isolated as pure culture by using standard microbiological method. One gram (1.00 g) each of dried soils was suspended in 99 ml sterile distilled water and serially diluted in sterile water up to 10^{-5}. An aliquot of 0.1 ml of each dilution was taken and spread evenly over the surface of starch casein agar (SCA) plate medium and incubated at 37 ^{0}C for 8 days. The pure isolates were obtained by repeated streaking on the freshly prepared microbiological media. Characterization and identification of the isolated microorganisms were based on their morphological and biochemical test (Agadagba, 2014).

2.3 Test microorganisms

The test microorganisms (*A. flavus* and *F. verticillioides*) were isolated from the orange soil's rhizosphere and maize soil's rhizosphere obtained from orange plantation in Ilale Area of Akungba Akoko and maize plantation in Adekunle Ajasin University's permanent site, Akungba Akoko, Nigeria. The pure cultures of the test microorganisms were obtained by using standard microbiological method. One gram (1.00 g) each of dried soils was suspended in 99 ml sterile distilled water and serially diluted in sterile water up to 10^{-7}. An aliquot of 0.1 ml of each dilution was taken and spread evenly over the surface of potato dextrose agar (PDA) plate medium and incubated at 28 ^{0}C for 3 days. The pure isolates were obtained by repeated streaking on the freshly prepared microbiological media. Fungal colonies were identified colonially using characteristics such as pigmentation on the surface and reverse, sporulation, mycelia and spore arrangement microscopically (Nyongesa *et al*., 2015).

2.4 Inoculum preparation

The microorganisms used were prepared according to McFarland turbidity standards. The suspensions were prepared by adding pure culture of the isolates into sterile distilled water in a test tube until it has the same turbidity as the prepared McFarland standard, which represent $1.5x10^{8}$ cfu/ml for bacteria and $1.5x10^{8}$ sfu/ml for fungi suspension. The absorbance of the suspension was then checked using a spectrophotometer at 623 nm and compared to McFarland turbidity standards solution. 0.1 ml

($1.5x10^7$) each of the suspensions (actinomycetes and fungi) were used for this research work (Cheesbrough, 2006).

2.5 Antagonistic effect against selected fungi

Antifungal activity of actinomycetes species against *A. flavus*, and *F. verticillioides* was determined by agar diffusion technique. For testing antifungal activity, potato dextrose agar (PDA) medium was used. After solidification of 9 cm Petri plate, agar surface was inoculated with antagonist actinomycetes. Then small disc from 5 days old fungal culture was taken by cork borer, each of 4 mm diameters. Each disc was cultured in the centre of each Petri dish to test the inhibition activity of each isolated actinomycetes. Each fungal growth was measured after 5 days of incubation at 28 °C. Fungal growth without bacterial inoculum was used as control. The cultures were examined for the presence of a clear inhibition zone around the mycelium discs (Sobia *et al.*, 2010).

2.6 Statistical analysis

The data obtained were analyzed. Data are presented as mean ± standard deviation. The significance of difference between different groups was tested using one-way analysis of variance (ANOVA) using SPSS Window 8 Version 23.

3.0 Results

3.1 Total actinomycetes counts from soil samples

The total actinomycetes counts isolated from different soil samples is represented (Table 1). The highest microbial count (10.00) was observed in the sample collected from Microbiology laboratory area while the least counts (2.33) was obtained in sample from Adekunle Ajasin University permanent

Table 1: Total Actinomycetes counts from soilsamples

Locations	SCA (cfu/g x 10^5)
APS	3.00 ± 0.58^{b}
ILA	6.00 ± 0.58^{d}
MLA	10.00 ± 0.00^{e}
AKU	2.33 ± 0.33^{a}
IBA	5.00 ± 0.00^{c}

Data are represented as mean ± standard deviation with the same superscript down the column is not significantly different ($p\leq0.05$).**Key**: **cfu/g** =colony forming units per grams; **APS**= Adekunle Ajasin University permanent site; **ILA=** Ilale, Akungba-Akoko; **MLA=** Microbiology Laboratory Area; **AKU**= Akua area, Akungba-Akoko; **IBA**= Ibaka area, Akungba-Akoko and **SCA**= starch casein agar.

3.2 Colony, cell morphological and biochemical characterization actinomycetes from soil samples

Table 2 shows the colony, cell morphological and biochemical characterization of actinomycetes from soil samples.

Table 2: **Colony, cell morphological and biochemical characterization of actinomycetes from soil samples**

Isolates codes	Cell morphology	Gram stain	Citrate	Motility	Catalase	Starch hydrolysis	Mannitol	Maltose	Glucose	Fructose	Sucrose	Lactose	Probable microorganisms
A	Cream, short chain	+R	+	-	-	+	-	+	+	+	+	+	*Actinomyces* spp
B	Cream, short cluster	+R	+	+	+	+	+	+	+	+	-	-	*Streptomyces* spp
C	Cream, short cluster	+R	+	-	+	-	+	+	+	+	+	+	*Rhodococcus* spp
D	Yellow, short chain	+R	+	-	+	-	-	-	-	+	+	+	*Norcadia* spp
E	Brownish white, short cluster	+R	+	-	+	+	-	-	-	-	-	-	*Mycobacterium* spp
F	Orange, short cluster	+R	+	-	+	-	-	-	-	-	-	-	*Streptomonospora* spp

Key: Positive= +; Negative= -; R= rod

3.3 The growth inhibitory activity of actinomycetes against *A. flavus* and *F. verticillioides* at five day

The growth inhibitory activity of actinomycetes against the *A. flavus* and *F. verticillioides* is represented (Table 4). The antagonistic potential among the actinomycetes showed that *Streptomyces* spp appeared the best against *A. flavus* and *F. verticillioides*.

Table 4: The growth inhibitory activity of *actinomycetes* against *A. flavus* and *F. verticillioides*

	Diameter of inhibition zones (mm)	
Actinomycetes	***Aspergillus flavus***	***Fusarium verticillioides***
Control	30.00 ± 0.01^{f}	10.00 ± 0.18^{c}
***Actinomyces* spp**	6.60 ± 0.04^{b}	8.60 ± 0.03^{b}
***Mycobacterium* spp**	6.80 ± 0.02^{b}	10.02 ± 0.10^{c}
***Rhodococcus* spp**	9.40 ± 0.03^{d}	14.30 ± 0.02^{e}
***Norcadia* spp**	8.10 ± 0.33^{c}	12.40 ± 0.11^{d}
***Streptomyces* spp**	16.00 ± 0.10^{e}	21.00 ± 0.05^{f}
***Streptomonospora* spp**	0.30 ± 0.01^{a}	0.70 ± 0.04^{a}

Data are represented as a mean ± standard deviation with the same superscript down the column is not significantly different ($p\leq0.05$).

4.0 Discussion

The impact of *A. flavus* and *F. verticillioides* on crops and livestock losses cannot be overemphasized. The aflatoxins produce by these microorganisms that cause a lot of adverse effect in lowering the yields of crops and the ability to cause various pathological conditions has led to widespread screening of foods and feeds potentially contaminated with them. This study is designed to evaluate the antifungal effect of actinomycetes isolated from different soil samples against these mycotoxigenic fungi. Different species of actinomycetes were isolated from the soil samples collected from five different areas in Akugba-Akoko, Ondo State. The result is similar to the findings of Kamara and Gangwar (2015), who isolated different species of actinomycetes from soil rhizospheric

of medicinal plant. This also corroborates the findings of Njenga *et al*. (2017), who reported of isolating different species of actinomycetes from soil sample.

Researchers have reported that 1g of soil sample contains about 10 billion microorganisms (4.2×10^6cfu/g) (dry weight) for bacteria species (Torsvik and Ovreas, 2002). The number of actinomycetes which is the highest bacterial counts (Microbiology laboratory area) obtained from all the samples collected in this study were lower than what has been reported. This might be due to the topography of the soil. The steepness of the terrain, exposure to the sun rays and lack of vegetation covering of the soil (bare) might have caused leaching of actinomycetes and therefore have partly contributed to the observed low actinomycetes cfu/g of dry soil.

In this study, actinomycetes especially *Streptomyces* spp possesses antagonistic effect against *A. flavus* and *F. verticillioides*. And this ability might be due to the fact that these microorganisms posses some hydrolytic enzymes or antibiotics against the mycotoxigenic fungi. *Streptomyces* spp has been reported to posses *Streptomyces* lytic activities that are mainly induced by lytic enzymes such as chitinase and glucanase (Matsumoto *et al*., 2006). And the major component of fungal cell wall is chitin, which is the substrate for chitinase enzymes (Hoell *et al*., 2006). However, the inhibition of fungi by *Streptomyces* spp might be due to the production of chitinase (Gohel *et al*., 2006).

5. Conclusion

In this study, it was discovered that actinomycetes (*Streptomyces* spp) has a better antagonistic effect against *A. flavus* and *F. verticillioides* by producing antifungal compounds that can reduce the losses encounter in the yields of crops by these fungi and provides a starting points. for discovering of new compounds that are environmental friendly, non-toxic and easily degradable than the chemical fungicides currently available. Hence, the used of actinomycetes against the mycotoxins produced by these fungi can be adopted instead of chemical fungicides.

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