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Influence of Growth Factors on *Aspergillus fumigatus* Antibiotic Production Efficacy

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ABSTRACT

The rise of antibiotic resistance poses significant clinical and public health challenges, resulting in treatment failures and increased mortality. Enteric bacterial diseases are a major concern, underscoring the urgent need for novel antibiotics from natural sources to combat resistant pathogens effectively.. This study investigates the influence of growth factors on A*spergillus fumigatus* antibiotic production efficacy A total of 100 soil samples were randomly and aseptically from different hospital dumping sites, and screened for the presence of *Aspergillus fumigatus* using appropriate mycological techniques. The fungal isolates were grown in a submerged culture, and screened for the production of antibiotics. *Aspergillus fumigatus* strain DTO402 (AFO402), *Aspergillus fumigatus* strain F7 (AFF7) and *Aspergillus fumigatus* strain KMM4631 (AFK4631) were isolated from the soil samples. The fungal isolates exhibited significant ($P<0.05$) production of inhibitory substances when the pH, temperature, carbon and nitrogen sources were 7.0, 25℃, extracted sugar from *Phoenix dactylifera* (PD) fruits and NOdZ (prepared mixture of *Rhizobium leguminosarum*, soybean meal and *Arachis hypogoea* nodule meal) respectively. This study demonstrates the potential of Aspergillus fumigatus strains (AFO402, AFF7, and AFK4631) isolated from hospital dumping sites as sources of novel antibiotics. Optimized growth conditions (pH 7.0, 25°C, Phoenix dactylifera sugar, and NOdZ nitrogen source) significantly enhanced antibiotic production, highlighting the promise of these fungal isolates in combating antibiotic-resistant enteric bacterial pathogens.

Keywords: Antibiotics, Mycological, Submerged, Strain, Inhibitory

INTRODUCTION

Filamentous fungi represent an important group of microorganisms known to synthesize a huge diversity of bioactive molecules that are known as secondary metabolites or natural antibacterial agents (antibiotics). Secondary metabolites are biologically active organic compounds that are not required for normal cell growth and metabolism but enable the organism to minimize competition. Secondary metabolites are used as medicines, flavourings, pigments, and recreational drugs (Newman *et al.,* 2012).

Research had revealed that a mold known as *Aspergillus fumigatus* has an ability to produce secondary metabolites otherwise known as antibiotics or antibacterial agents which exert a bactericidal effect on human pathogens such as sorbitol negative *Escherichia coli* that infects the gastrointestinal tract. The genus *Aspergillus* are highly ubiquitous saprophytic molds that thrive in diverse environment. Certain *Aspergillus* such as *Aspergillus fumigatus* are also pathogenic and infect human lungs especially the immune-compromised individuals.

It had been reported that about 10,000-15,000 liters of air that a typical person inhales each day are estimated to contain a few hundred *A. fumigatus* conidia. The conidia are 2-3 µm in diameter and can therefore penetrate deep into the lungs, reaching the alveoli. *A. fumigatus* is also heat tolerant, resistant to oxidative stress, has a high growth rate, and can survive on various nutrients (Grice *et al.*, 2013).

MATERIALS AND METHODS

Collection of samples: A total of 300 soil samples from hospital waste dumping site were randomly collected from different sites in Ihiala L.G.A, Anambra State. This was carried out using the method described in the study published by Iheukwumere *et al.* (2021). The litter from the soil surfaces was carefully scrapped out using sterile stainless spoon. The soil auger was derived to a plough depth of 15 cm in the farm land, and soil sample was drawn up to 10 samples from each sampling unit into a sterile tray. The samples were thorough mixed and foreign materials such as roots, stones, pebbles and gravels were carefully removed. The soil sample was then reduced to half by quartering the sample. Quartering was carried out by dividing the soil sample into four equal parts and the two opposite quarters were discarded and the remaining two quarters were mixed. The process was repeated for the rest of soil samples

used for this study. The samples were carefully labeled and then kept in a disinfected cooler, to maintain its temperature and stability of the number of the isolates. The samples were transported to the laboratory for analysis.

Isolation of the Fungal Isolates: The media used for this isolation was Sabouraud dextrose agar (SDA/BIOTECH). One gram of the soil sample was weighed into boiling test tube; 5 mL of normal saline was added and shake thoroughly and then make up to 10 mL using the normal saline (10^{-1}dilution). One milliliter of the suspension was added to four milliliter (4 mL) of normal saline (0.85% NaCl), which was give 5^{-1} dilution. From 5^{-1} dilution test tube, a five-fold serial dilution was carried out to obtain 5^{-5} dilution. One milliliter aliquot from 10^{-1}, 5^{-1} and 5^{-5} test tubes were collected and aseptically plated onto solidified sabouraud dextrose agar plate (90 mm x 15 mm) which was prepared according to the manufacturers instruction and the procedures described in Cheesbrough (2010) supplemented with chloramphenicol (0.05 %) and spread using a spreading rod. The SDA was incubated in an inverted position for 5-7 days at 30 ± 2^0C.

Identification of Fungal Isolates: The fungal isolates were identified to the genus/species level based on macroscopic, microscopic and molecular characteristics of the isolates obtained from pure cultures as described in the study published by Iheukwumere *et al*. (2020).

Screening the fungal isolates for antibiotic production: For antibiotic production, Mueller Hinton Agar (MHA) medium was prepared according to the manufacturer's direction. This was allowed to cool and then poured in Petri dishes and kept in incubator at 37^0C for 24 h to check its sterility. Then the test organisms; *Escherichia coli, Staphylococcus aureus,* and *Salmonella species* were grown on broth culture at 37^0C for 24 h. After incubation, sterilized swab stick was dipped into the broth cultures and swabbed on MHA plates and allowed for 1 h. Then wells were made on the MHA plates using sterile cork borer. Then the broth culture of the fungal isolates were carefully centrifuged at 6000 rpm for 10 minutes and their supernatants were poured in the wells and incubated at 37^0C for 48 h. zones of inhibition was observed after incubation (Adeel *et al.,* 2017).

Extraction of Antibiotics: The characterized fungal isolates were grown in a Brain heart Infusion broth:

10g/L,peptone 5g/L, dextrose 5g/L, NaCl 5g/L, Na2HPO4, 2.5g/L, (NH4)2SO4 1g/L, CaCl2 0.02g/L, KH2PO4, 15g/L, yeast extract 5g/L, starch 1g/L, cysteine HCl and 1g/L, MgSO4 0.2g/L. This was incubated at room temperature (30±2°C) for 7 days with intermittent manual shaking. In order to study the definite growth pattern of the isolates and optimum antibiotic production, the temperature, pH, carbon and nitrogen sources were optimized for each strain of the *Aspergillus* species (Adeel *et al.,* 2017)

pH optimization: The characterized fungal isolates were grown in a Brain heart Infusion broth: 10g/L, peptone 5g/L, dextrose 5g/L, NaCl 5g/L, Na2HPO4, 2.5g/L, (NH4)2SO4 1g/L, CaCl2 0.02g/L, KH2PO4, 15g/L, yeast extract 5g/L, starch 1g/L, cysteine HCl and 1g/L, MgSO4 0.2g/L. adjusted at varying pH range (4.0, 5.0, 6.0, 7.0, 9.0, 10.0) prepared in triplicates, and incubated at room temperature (30±2°C) for 7 days (Adeel *et al.,* 2017)

Temperature optimization: The characterized fungal isolates were grown in a Brain heart Infusion broth 10g/L,peptone 5g/L, dextrose 5g/L, NaCl 5g/L, Na2HPO4, 2.5g/L, (NH4)2SO4 1g/L, CaCl2 0.02g/L, KH2PO4, 15g/L, yeast extract 5g/L, starch 1g/L, cysteine HCl and 1g/L, MgSO4 0.2g/L. adjusted at optimum pH prepared in triplicates, and incubated at varying temperatures(25°C, 30°C, 35°C,50°C) (Adeel *et al.,* 2017)

Effect of nitrogen sources on antibiotic production: The characterized fungal isolates were grown in a Brain heart Infusion broth 0.5% of varying nitrogen sources [(NH4)2SO4, peptone, $NaN0_3$, $KN0_3$], 5g/L, dextrose 5g/L, NaCl 5g/L, Na2HPO4, 1g/L, CaCl2 0.02g/L, KH2PO4, 15 g/L, yeast extract 5g/L, starch 1g/L, cysteine HCl and 1g/L, MgSO4 0.2g/L adjusted at optimum pH prepared in triplicates, and incubated at optimum temperature (Adeel *et al.,* 2017)

Effect of simple and carbon sources on antibiotic production: The characterized fungal isolates were grown in a Brain heart Infusion broth containing 1.5% of varying carbon sources (glucose, sucrose, lactose, starch maltose), 10g/L, NodZ ,5g/L, NaCl 5g/L, Na2HPO4, 2.5g/L, (NH4)2SO4 1g/L, CaCl2 0.02g/L, KH2PO4, 15g/L, yeast extract 5g/L, starch 1g/L, cysteine HCl and 1g/L, MgSO4 0.2g/L adjusted at optimum pH prepared in triplicates, and incubated at optimum temperature (Adeel *et al.,* 2017)

Antibiotic production at optimum conditions: The characterized fungal isolates were grown in a Brain heart

Infusion broth: 10g/L,peptone 5g/L, glucose 5g/L, NaCl 5g/L, Na2HPO4, 2.5g/L, (NH4)2SO4 1g/L, CaCl2 0.02g/L, KH2PO4, 15g/L, yeast extract 5g/L, starch 1g/L, cysteine HCl and 1g/L, MgSO4 0.2g/L. adjusted at pH 7.0 prepared in triplicates. This was incubated at room temperature (30±2°C) for 7 days with intermittent manual shaki (Adeel *et al.,* 2017)

Data Analysis: The data obtained in this study were presented in tables and figures. Their percentages were also calculated. Significance of the study was carried out using one way Analysis of Variance (ANOVA) at 95% confidence level. Pair wise comparison was carried out using student "t" test (Iheukwumere *et al*., 2018, Iheukwumere *et al*., 2020).

RESULTS

The macroscopic and microscopic characterization of the fungal isolates are presented in Tables 1 and 2. The results showed that the fungal isolates initially appeared white on SDA within 2-3 days while gray-green with white edges was observed later within 5 days. The reverse colour of the isolates was pale and light yellow, and the growth rate was also rapid. The texture of the colony appeared cottony and woolly while the colour of the mycelium appeared gray-green. Similarly, the microscopic features of the fungal isolates showed septate hyphae and gray-green conidia. The shape of the conidia appeared ellipsoidal and the vesicle showed globose appearance. The molecular characteristics of the fungal isolates are presented in Table 3. The features showed that the fungus was *Aspergillus fumi01gatus* of different strains as showed in Table 3.

The effects pH, temperature, carbon source, and nitrogen source on antibiotics production are presented in Tables 4-15. The result revealed that pH range of 6.0 – 8.0 supported optimum production of antibiotics against the food pathogens tested while low production of antibiotics was recorded at pH of 4.0, 5.0, and 9.0. The result also revealed that the highest antibiotics were produced at low temperature of 25ºC while the lowest production of antibiotics was recorded at 40ºC and 45ºC. The effect of carbon source on the production of antibiotics showed that highest antibiotics were produced when date-sugar was used as a carbon source followed by glucose while sucrose yielded the lowest antibiotics. The effect of nitrogen source on the production of antibiotics revealed that Nod Z supported the production of highest antibiotics followed by soybean meal while the lowest antibiotics were produced when peptone was used as a nitrogen source.

Table 1: Macroscopic characteristics of the fungal isolates

Parameter	Isolate V	Isolate U	Isolate W
Initial Appearance on SDA(2-3 days)	White	White	White
Later Appearance on SDA(5 days)	Gray-green with white edges	Gray-green with white edges	Gray-green
Reverse Colour	Light Yellow	Pale	Pale yellow
Growth Rate	Rapid	Rapid	Rapid
Colony Texture	Cottony	Wooly	Wooly
Colour of Mycelium	Gray-green	Gray-green	Gray-green
Fungus	Aspergillus species	Aspergillus species	Aspergillus species

Table 2: Microscopic characteristics of the isolates

Parameter	Isolate V	Isolate U	Isolate W
Nature of hyphae	Septate	Septate	Septate
Colour of Conidia	Gray-green	Gray-green	Gray-green
Conidia head	Columnare	Columnar	Columnar
Shape of Conidia	Ellipsoidal	Ellipsoidal	Ellipsoidal
Shape of Vesicle	Globose	Globose	Globose
Colour of Conidiophore	Hyaline	Hyaline	Hyaline

Texture of Conidiophore	Smooth	Smooth	Smooth
Length of Conidiophore	Short	Short	Short
Seriation (Sterigmata)	Uniseriate	Uniseriate	Uniseriate
Fungus	Aspergillus fumigatus	Aspergillus fumigatus	Aspergillus fumigatus

Table 3: Molecular characteristics of the fungal isolates

Parameter	Isolate V	Isolate U	Isolate W
Max Score	1644	2442	2929
Total Score	1644	2442	2929
Query Cover(%)	100	100	100
E-Value	0.0	0.0	0.0
Identity(%)	100	100	100
Accession Number	MT316338	KR023997	OR578448
Description	*Aspergillus fumigatus* strain DTO402(AFD402)	Aspergillus fumigatus strain F7(AFF7)	*Aspergillus fumigatus* strain KMM4631(AFK4631)

Table 4: Effects of pH on the production of antibiotics from AFK4631

ph	STWG	STCM	STER12
4.0	11.60	12.71	12.11
5.0	13.80	14.20	13.96
6.0	17.21	18.34	17.92
7.0	19.33	21.46	20.03
8.0	18.81	21.22	20.01
9.0	9.13	11.02	11.26

Table 5: Effects of pH on the production of antibiotics from AFD402

pH	STWG	STCM	STER12
4.0	9.30	12.06	10.80
5.0	10.88	13.96	11.96
6.0	14.21	15.36	15.02
7.0	17.02	19.81	18.04
8.0	16.80	19.20	17.84
9.0	8.10	10.10	9.06

Table 6: Effects of pH on the production of antibiotics from AFF7

pH	STWG	STCM	STER12
4.0	12.17	15.42	13.42
5.0	14.21	16.74	14.92
6.0	19.30	21.22	19.72
7.0	22.12	26.81	24.02

8.0	20.13	24.16	20.15
9.0	12.05	13.44	12.18

Table 7: Effects of temperature on the production of antibiotics from AFK4631

Temperature(ºC)	STWG	STCM	STER12
25	19.76	21.94	20.28
30	16.40	19.06	17.36
35	12.30	14.26	12.82
40	8.92	10.38	9.47
45	7.12	8.04	7.86

Table 8: Effects of temperature on the production of antibiotics from AFD402

Temperature(ºC)	STWG	STCM	STER12
25	17.29	20.02	18.86
30	14.42	16.04	14.92
35	11.08	12.43	12.08
40	8.06	9.18	8.80
45	7.10	7.81	7.42

Table 9: Effects of temperature on the production of antibiotics from AFF7

Temperature(ºC)	STWG	STCM	STER12
25	22.88	26.91	24.34
30	19.22	21.62	20.08
35	15.44	17.54	18.26

40	8.15	8.64	9.52
45	7.30	7.90	7.50

Table 10: Effects of carbon source on the production of antibiotics from AFK4631

Carbon Source	STWG	STCM	STER12
Glucose	19.82	21.92	20.42
Fructose	18.86	20.46	20.06
Maltose	17.64	19.14	19.02
Sucrose	16.36	18.66	18.14
Date-Sugar	20.42	22.81	21.37

Table 11: Effects of carbon source on the production of antibiotics from AFD402

Carbon Source	STWG	STCM	STER12
Glucose	17.46	19.87	18.62
Fructose	16.81	18.26	17.26
Maltose	13.08	15.08	14.38
Sucrose	11.21	12.92	11.64
Date-Sugar	18.02	20.18	18.82

Table 12: Effects of carbon source on the production of antibiotics from AFF7

Carbon Source	STWG	STCM	STER12
Glucose	22.66	26.88	24.92
Fructose	15.15	21.22	19.32
Maltose	13.42	16.08	16.72

Sucrose	11.13	14.26	13.54
Date-Sugar	22.86	27.21	25.18

Table 13: Effects of nitrogen source on the production of antibiotics from AFK4631

Nitrogen Source	STWG	STCM	STER12
Peptone	16.76	18.74	17.06
NaNO3	19.88	21.36	20.64
$(NH_4)_2SO_4$	18.20	19.82	19.26
Beef Extract	18.08	19.76	19.12
Soybean Meal	19.36	21.08	20.48
Nod Z	20.66	22.84	21.32

Table 14: Effects of nitrogen source on the production of antibiotics from AFD402

Nitrogen Source	STWG	STCM	STER12
Peptone	15.46	15.88	15.76
$NaNO_3$	18.22	19.92	18.82
$(NH_4)_2SO_4$	17.66	17.62	18.36
Beef Extract	17.64	17.22	18.24
Soybean Meal	18.04	19.20	18.64
Nod Z	18.86	20.48	19.76

Table 15: Effects of nitrogen source on the production of antibiotics from AFF7

Nitrogen Source	STWG	STCM	STER12
Peptone	19.32	18.66	19.67

$NaNO_3$	23.46	26.88	24.61
$(NH_4)_2SO_4$	19.88	22.42	23.15
Beef Extract	19.48	21.31	21.92
Soybean Meal	23.06	26.41	23.92
Nod Z	23.82	27.64	24.98

DISCUSSION

Aspergillus species isolated from various soils have been found to produce antibacterial, antifungal and anti tumour metabolites. Species of *Aspergillus* are known to produce mycotoxin, organic acids and antibiotics. *A. fumigatus* is an especially prolific producer of secondary metabolites such as fumiginin (Abdel-Aziz *et al.,* 2017).

The characteristic features of *Aspergillus fumigatus* strain DTO402 (AFD402), *Aspergillus fumigatus* strain F7 (AFF7) and *Aspergillus fumigatus* strain KMM4631 (AFK4631) isolated from garden soil in the present study supported the reports of Naqvi *et al*. (2013) and Jeanvoine *et al*. (2017) who studied various soil samples for an antibiotic-producing *Aspergillus* species. The cultural features and morphology of the *Aspergillus* species isolated in their studies confirmed the observation made in this study concerning *Aspergillus* species. However, the previous researchers isolated other species of Aspergillus such as *niger, flavus*, and *fumigatus*, though their strains were not fully elucidated as was carried out in the present study.

This study showed that optimum antibiotics were produced at pH range of 6.0 – 8.0. This observation agrees with the work reported by Shikuku *et al*. (2013) who investigated the effect of pH, carbon, and nitrogen sources on antibiotic production by actinomycetes isolates from River Tana and lake Elementaita, Kenya. They may have developed metabolic characteristics that enable them to thrive and effectively combat bacteria in their preferred pH conditions. Similarly, the optimum antibiotics produced at a temperature of 25ºC agrees with the findings of Jain and Pundir (2011) who studied the effect of fermentation medium, pH and temperature variations on antibacterial soil fungal metabolite production but disagrees with the report of

Svahn and Bjorklund (2015) who stated that certain antibiotics are thermally stable at moderate temperature range of 50-250 °C. The ability of the fungal isolates to produce optimum antibiotics using date-sugar could be attributed to its excessive rich in sweet carbohydrate, though sucrose yielded the lowest antibiotic. This observation disagrees with the findings of Adeyemo *et al*. (2020) who discovered that the presence of simple sugars such as glucose inhibited the production of antibiotics. In another study conducted by Shikuku *et al*. (2013), sucrose was the preferred carbon source, followed by urea and fructose.

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