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In-vitro Biocontrol Activity of a Novel Soil Strain *Streptomyces albidoflavus* Against *Fusarium oxysporum* as Causal Agent of Fusarium wilt in Banana Plants

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ABSTRACT

Fusarium oxysporum f. sp. *Cubense* is causal agent of *Fusarium* wilt disease in Banana. The activity of antagonistic bacterial strain was studied for the low-cost and eco-friendly management of *F. oxysporum* in banana. *Streptomyces* S128 was identified by 16S rRNA sequence analysis and resulted as *Streptomyces albidoflavus*. Bioassay activity showed inhibition diameter of 22.5mm against *Fusarium oxysporum* f. sp. *Cubense*. Optimum combination of factors in the fermentation medium was obtained as: soluble starch 4%, peanut flour 2%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.8%, NaCl 0.8%. Identification of active compounds were performed via GC/MS chromatographic techniques. There were total of nineteen compounds identified in the extract. On the base of area percent (18.10) Phenol, 2, 4-bis (1, 1-dimethylethyl) and (18.71) morphine were the major constituents of extract. This study concluded that *Streptomyces albidoflavus* effective against *Fusarium oxysporum* f. sp. Cubense, which might be introduced as an effective biocontrol agent for sustainable agriculture.

KEY WORDS: BANANA FUSARIUM WILT, STREPTOMYCES ALBIDOFLAVUS, GC-MS, MORPHINE.

INTRODUCTION

Over 400 million people rely on Banana (*Musa* spp.) as major subsistence (Dale et al. 2017). Fusarium wilt or Panama is one of the destructive diseases of banana. In the past century in the early days, it has caused heavy economic loss (Ploetz 2015). The production of Banana (*Musa* spp.) is severely in danger because of the infection of the soil-borne fungus q f. sp. cubense (Foc) and is commonly referred to as Panama disease (Dita et al. 2018). Due to complex nature of soil zone, it's quite challenging to combat soil-borne diseases. Soil-borne fungus had severe effects on crops which leads to heavy economic loss (Jayaprakashvel et al. 2019). Chemical practices in disease management of Fusarium wilt can make the soil unfit (Siamak and Zheng 2018). It also caused negative effects on health, so biocontrol is an alternative measure (Rajaofera et al. 2019). In several mechanisms of disease management, this approach has gained significant value, (Bubici et al. 2019).

Currently, the demand for natural bioactive substances has increased because of the potential effects in clinical practices and as well as in crop protection (Singh et al. 2017). In recent years, bioactive compounds are in high demand in the pharmaceuticals and naturopathy, due to their health benefits to human and plants. *Actino* bacteria are a major source to obtain novel compounds, which could be utilized in clinical practices, pharmaceutical industry and agricultural applications, (Barka, et al. 2016; Chater 2016). Mostly *Actinomyces* obtained have been from the soil (Guo et al. 2015) as production of natural compounds from microbes requires optimal growth conditions besides the nutrient medium (Rajnisz et al. 2016).

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Nowadays different statics models are applied to optimize the fermentation mechanisms to extend the production of bioactive compounds, (Latha et al. 2017). There are several approaches in use, such as GC-MS, LC-MS, and NMR, for identification and structural characterization of the bioactive compounds (Tiwari et al. 2015). GC-MS is an innovative approach to identify the compound (Awla et al. 2016). In the current study, we have identified a novel potent *Streptomyces* strain for the biological control of banana *Fusarium* wilt. This study was aimed to produce secondary metabolites by optimizing the nutrient medium through Orthogonal Array Design, extraction and identification of bioactive compounds by GC-MS.

MATERIAL AND METHODS

Microorganisms: All the microbes, Banana *Fusarium* wilt pathogen and bio-agent were obtained from plant pathology lab, College of Plant Protection, Shenyang Agricultural University, China. *Streptomyces* strain 128 and other comparative antagonistic microbes (*Bacillus subtilis, Trichoderma harzianum, Paenibacillus polymyxas, Bacillus licheniformis, Streptomyces cacaoi, Bacillus laterosporus*, and *Bacillus mucilaginosus*), were maintained on nutrient and Gausses medium. *Fusarium oxysporum* was maintained on PDA.

Identification of Streptomyces strain: PCR amplification of the 16S rRNA gene of strain S128 was performed using the universal primer: 27(5'AGTTTGTCMTGGCTCAG-3) and 1492R (5′-GGTTACCTTACGACTT-3) (verity TM 96-well PCR, Applied Biosystems, Singapore). The PCR products were sent to Sangon Biotech (Shanghai, China) Co., Ltd for sequence determination. Phylogenetic analysis was conducted by using Mega version 6 (Ahsan et al. 2017).

Inoculum development: Fermentation was performed in two stages, seed growth and production of the active antifungal substance. Strain S128 was grown on plates of gausses medium (Gao et al. 2016) at 28 °C for 5 days after spore production in the liquid fermentation medium. Two spore cakes (5 mm) of Strain 128 were used to inoculate 40ml medium in a 250 mL flask volume and incubated at 28 °C with a shaking speed of 160 rpm for 48 h.

Fermentation process: From seed culture, 5% (v/v) were inoculated aseptically into 250 mL flask containing 40 mL of fermentation medium The medium comprised of [47 g soluble starch, 3 g yeast extract, 22 g peanut meal, 2.7 g $(NH_4)_2SO_4$, 2.7 g NaCl, 2.7 g $CaCO_3$ dissolved in 1 L distilled water and pH were adjusted to 6.8–7.2] and incubated at 28 °C in a rotatory shaker (HZQ-F16 Harbin Dong Lian Electronic Technology Production. Co., Ltd., China) at the speed of 160 rpm for 96 h. After that, the fermented culture was centrifuged the supernatant was stored at 4 °C for further study. The antifungal activity was determined by measuring the diameter of inhibition zones (Ahsan et al. 2017).

Experimental design for optimization of nutrients: The main nutrient factors affecting the fermentation of the strain and its concentration range were determined by a single factor test. On the basis of this, the nutritional formula of the strain was optimized by orthogonal design test. Based on the average value, the optimal fermentation medium formulation was determined by orthogonal analysis.

Effect of KH_2PO_4 on antibiotic production: KH_2PO_4 was added in the optimized medium in the amounts of 0.01%, 0.02%, 0.03%, and 0.04%, respectively, without KH_2PO_4 as control, and cultured at 28 °C, 140 r / min constant temperature shaker for 96 h. The Oxford cup plate method was used to determine the antifungal activity of the fermentation broth.

Effect of inorganic salts and trace elements on bioactive compound production: On the basis of optimizing the medium, a certain amount of inorganic salts and trace elements were added respectively, that is, K_2SO_4, ZnSO4, $MnSO_4$, $MgSO_4$, $CuSO_4$, and $FeSO_4$ were added with a concentration of 0.01%, 0.02%, 0.04%, and 0.08%, respectively, at 28 °C and 140r/min shaking speed for 96 hours. The antifungal activity of the fermentation broth was determined by Oxford cup plate method and without adding trace elements was considered as a control value.

Biochemical profiling for stability test of fermentation broth: Using the dinitrosalicylic acid, reducing sugar in the fermentation batch was measured and total sugars were measured by Phenol–sulphuric acid method. Amino nitrogen was determined using ninhydrin reagent. pH values of the fermentation batch were determined at different interval of time using pH meter. Dry cell weight analysis was done by the method of (Ahsan et al. 2017).

Separation, extraction, and identification of active compounds: The fermented broth was further treated with different organic solvents by the solvent extraction method. Four different solvents were used to separate fermented broth, i.e. Ethyl acetate, Diethyl ether, Ethanol, Methanol, and N-Butanol, with 1:1ratio and check the activity. Potent separated extract further purified by Silica gel column chromatography. The column was packed with silica gel (60–120 mesh). The sample to be separated was loaded on the packed column and eluted with the ethanol solvent at the flow rate of one drop per 30 seconds. Collected the fractions in test tubes and check the antifungal activity of the fractions. Most potent fraction selected to identify the active compound. GC-MS was used to identify the active compound (Ahsan set al. 2017).

Antifungal assay: Purified Silica gel column chromatography fractions were utilized for antifungal activity of *Streptomyces* against the *Fusarium oxysporum* f. sp. Cubense. Make a serial dilution concentration of most potent fraction, i.e., 0.5, 2, 4, 6, 8, 10 µg/ml. Oxford cup method was used.

Effects of inhibition rate among *S. albidoflavous* and other biocontrol agents against *Fusarium oxysprum*: A comparative antifungal activity was conducted against *Fusarium*. *S. albidoflavnous* and other test biocontrol agents (*Bacillus subtilis, Trichoderma harzianum, Paenibacillus polymyxas, Bacillus licheniformis, Bacillus laterosporus*, and *Streptomyces cacaoi* were evaluated against *Fusarium* wilt. Antifungal activity was performed by oxford cup plate method.

Statistical analysis: Statistical analysis was performed with Minitab software version 0.7.

Table 1 Results of L_{25} (5^6) orthogonal test for the production of antifungal compounds

Run No.	Peanut flour (%)	Soluble starch (%)	Sodium chloride (%)	Yeast extract (%)	$(NH_4)_2SO_4$ (%)	$CaCO_3$ (%)	Diameter of inhibition zone (mm)
1	0	0	0.2	0.4	0.6	0.2	9.51
2	2	0	0. 6	0.8	0.8	0.8	19.21
3	4	0	0	0.6	0	0.6	22.20
4	6	0	0.4	0	0.4	0	10.51
5	8	0	0. 8	0.2	0.2	0.4	25.55
6	0	2	0.6	0.6	0.4	0.4	22.01
7	2	2	0	0	0.2	0.2	31.11
8	4	2	0.4	0.2	0.6	0.8	31.10
9	6	2	0.8	0.4	0.8	0.6	25.09
10	8	2	0.2	0.8	0	0	22.81
11	0	4	0	0.2	0.8	0	0
12	2	4	0.4	0.4	0	0.4	21.98
13	4	4	0.8	0.8	0.4	0.2	25.00
14	6	4	0.2	0.6	0.2	0.8	23.90
15	8	4	0.6	0	0.6	0.6	25.10
16	0	6	0.4	0.8	0.2	0.6	21.76
17	2	6	0.8	0.6	0.6	0	20.45
18	4	6	0.2	0	0.8	0.4	23.95
19	6	6	0.6	0.2	0	0.2	17.00
20	8	6	0	0.4	0.4	0.8	17.50
21	0	8	0.8	0	0	0.8	15.14
22	2	8	0.2	0.2	0.4	0.6	11.80
23	4	8	0.6	0.4	0.2	0	11.00
24	6	8	0	0.8	0.6	0.4	10.00
25	8	8	0.4	0.6	0.8	0.2	8.00
k1	17.510	13.780	18.630	16.800	18.852	17.880	
k2	26.467	20.910	18.413	19.801	14.976	22.398	
k3	18.824	22.400	16.400	19.087	19.818	20.395	
k4	20.150	17.160	18.775	21.274	17.042	12.989	
k5	10.756	19.912	21.896	16.898	23.356	20.875	
k_{max} - k_{min}	15.654	8.616	5.431	4.980	8.340	9.547	

RESULTS AND DISCUSSION

Identification of Streptomyces strain: A partial 16S rRNA gene sequence (1435 nucleotides) of strain S128 was determined and deposited in the Gene Bank database (Waiting for Accession number). Comparative 16S rRNA gene sequence analysis using BLAST showed that the strain could be classified as a member of the genus *Streptomyces* and shared sequence identity (99%) with *Streptomyces albidoflavus* Eu257268. A 16sRNA gene-based phylogenetic tree was constructed with the maximum likelihood method with the different *Streptomyces* reference species available in the Gen Bank database (Fig-1). Phylogenetic analysis indicated that strain S128 closely clustered with the strain *Streptomyces albidoflavus* (Eu257268).

Submerged fermentation: Based on the single factor optimization, the number of influencing factors and the different levels of each factor was determined. In order to eliminate the interaction between various factors and the differences between different test batches, and determine the optimal ratio of various factors in the fermentation medium, soluble starch, peanut cake powder, sodium

chloride, yeast extract, ammonium sulfate and carbonic acid Calcium was the most influencing factor. Orthogonal design test L25 (56), results were mentioned in (Table 1). Based on the results of 3 trials, the optimal fermentation medium formulation was determined by orthogonal analysis.

In the table, ki (i = 1, 2, 3, 4, 5) represents the average value of the diameter of the inhibition zone of the fermentation broth when the i-th level of a factor is combined with other factors, and the highest ki value is selected as the optimal level of the factor, that is, if a factor k2>k1, k3, k4 and k5, the second level of the factor is selected as the optimal fermentation level. So the best combination of factors in the fermentation medium was obtained as: soluble starch 4%, peanut flour 2%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.8%, NaCl 0.8%. Kmax-kmin represents the extreme difference between the average values of different factors, and its size represents the degree of influence of different factors on the antibacterial activity of the fermentation broth. An increase in the values; cause an increase the degree of influence.

Conversely, the smaller the values decrease the degree of influence. The smaller, so it can be seen that the degree of influence of the six factors on the activity of the fermentation broth is: peanut flour > calcium carbonate > soluble starch > ammonium sulfate > sodium chloride > yeast powder. The shake flask fermentation test was carried out with the best medium formula and the original medium formula under the same culture conditions. The results showed that the diameter of the inhibition zone of the optimized fermentation broth was higher than that of the original medium %.

Table 2. Effect of mineral salt and trace element on yield of *S. albidoflavus*

Concentration (%)	Diameter of inhibition zone (mm)					
	$K2SO_4$	$ZnSO_4$	$MnSO_4$	$MgSO_4$	$CuSO_4$	$FeSO_4$
0(CK)	30.54	29.10	28.54	28.98	25.58	29.14
0.01	28.38	27.3	28.62	27.92	0	28.70
0.02	27.60	26.62	28.82	27.78	0	28.24
0.04	27.36	0	30.24	27.12	0	28.00
0.08	27.36	0	30.24	28.88	0	28.00

Table 3. Metabolism of *S. albidoflavus* during fermentation in shaking flasks

Parameter	Culture time (h)										
	0	12	24	36	48	60	72	84	96	108	120
Total sugar (mg/mL)	84.7	78.5	76.1	64.2	50.7	40.3	34.3	27.6	19.8	14.2	11.3
Reducing sugar (mg/mL)	0	3.2	4.8	5.6	15.7	18.4	20.3	17.5	14.3	8.9	8.3
Amino nitrogen (mg/mL)	0.63	0.61	0.62	0.59	0.56	0.50	0.38	0.15	0.12	0.11	0.09
pH value	6.4	6.5	6.5	6.5	6.5	6.5	6.1	5.9	5.9	5.3	5.3
Dry mycelium weight (mg/mL)	0	4.1	6.3	14.5	19.3	20.9	20.9	22.4	21.7	19.5	18.1

Effect of inorganic salts and trace elements on the yield of active compound: Microorganisms require certain inorganic salts, and trace elements such as iron, magnesium, zinc, manganese and potassium during growth and reproduction and secondary metabolite synthesis. The effect of many metal ions on the physiological activity of microorganisms is related to their concentration, low concentrations tend to be stimulating, and high concentrations show inhibition. In order to further determine whether the trace elements contained in the fermentation medium of *Streptomyces* S128 can meet the needs of the synthesis of secondary metabolites, six different inorganic salts such as K_2SO_4, $ZnSO_4$, $MnSO_4$, $MgSO_4$, $CuSO_4$, and $FeSO_4$ were added to the optimized medium to study the pair of elements and impact of compound production.

The results showed that the yield of the bioactive compounds was increased after adding proper amount of $MnSO_4$ in the medium; while the yield of agricultural anti-SNO_3 was decreased after adding $ZnSO_4$, when the content exceeded 0.02%, the fermenting cells could not produce the bioactive compound. After adding $CuSO_4$, the bacteria could not produce compound; after adding $FeSO_4$ and K_2SO_4, the capacity of the cells was reduced to some extent; after adding $MgSO_4$, there was no effect on the yield of extract (Table 2).

Biochemical changes during fermentation: Biochemical analysis during fermentation process showed a stable production of secondary metabolites. Results showed in (Table 3), after inoculation to the fermentation medium, the reducing sugar content increases with the degradation of the starch. After that, the content of reducing sugar

decreased due to the growth of mycelium and energy activities such as metabolism. At the same time, carbon sources and nitrogen sources were continuously consumed, and the total sugar content and amino nitrogen content also showed a downward trend.

Table 4. GC-MS chromatograph of identified compounds from the extract of *S. albidoflavus*

Peak #	Retention time	Area %	Name of the Compound	Chemical Formula	Molecular Weight
1	4.563	3.9	Stannane, trimethyl propyl-	$C_5H_{14}Sn$	192.87
2	4.845	4.1	Nonadecane	$C_{19}H_{40}$	268.5
3	8.030	7.110	1,3,5,7,9-pentaethyl cyclopentasiloxane	$C_{10}H_{25}O_5Si_5$	365.73
4	15.327	5.21	Tetracosane, 1-bromo-octadecane	$C_{24}H_{49}Br$	417.5
5	17.372	18.10	Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{16}H_{26}O_3$	266.381
6	17.895	0.810	Benzyl alcohol, .alpha.- (1-aminoethyl)-m-hydroxy	$C_9H_{14}ClNO_2$	203.66
7	18.394	1.06	Hexadecanoic acid , methyl ester	$C_{17}H_{34}O_2$	270.4507
8	18.841	0.63	Pinacolyl alcohol, TMS derivative	$C_6H_{14}O$	102.174
9	18.929	2.01	3H-pyrazol -3-one, 2,4 –dihydro-2, 5-diphenyl	$C_{15}H_{14}N_2$	222.28
10	19.258	0.98	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O9Si_9$	666
11	20.086	6.3	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$	278
12	20.504	18.71	Morphinan, 7, 8-dihydro	$C_{17}H_{19}NO_4$	301.342
13	20.351	5.31	Dibutanyl morphine	-	-
14	21.643	1.25	2-Ethylacridine	$C_{18}H_{19}N_3O$	293.4
15	22.689	1.98	Benzoic acid, 2, 5-bis (trimethylsiloxy) -, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370.66
16	22.830	6.96	Phenol, 1`,1 dimethyl-ester	$C_8H_{10}O_3$	154.16
17	23.917	2.45	Benzoic acid, 2,3 methyl-ester	$C_{11}H_{14}O_2$	178.23
18	25.104	6.00	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222.46
19	26.485	2.5	Tetracosane, 1-bromo-octadecane	$C_{18}H_{37}Br$	333.4

Table 5. Inhibition Effects of *S. albidoflavus* and Test Antagonistic agents against *Fusarium oxysporum* f. sp. Cubense

Antifungal Biocontrol Agents	Concentration (Cfu/ ml)	Colony diameter (mm)	Inhibitory rate (%)	Significance of Differences	
				5%	1%
Bacillus subtilis	1.0×10^{9}	9.92	92.65	a	A
Streptomyces albidoflavus	1.0×10^{9}	10.79	89.53	a	A
Trichoderma harzianum	1.0×10^{9}	29.88	71.42	b	B
Paenibacilluspolymyxas	1.0×10^{9}	22.97	83.09	bc	BC
Bacillus licheniformis	1.0×10^{9}	26.76	74.97	bcd	BCD
Streptomyces cacaoi	1.0×10^{9}	13.07	91.83	cd	CD
Bacillus laterosporus	1.0×10^{9}	19.56	80.11	cd	CD
Bacillus mucilaginosus	1.0×10^{9}	13.00	91.00	d	D

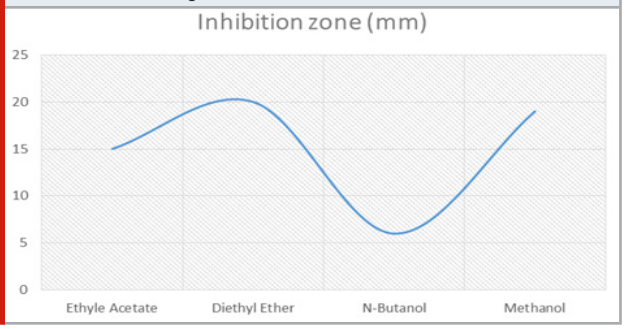
The concentration of the bacteria increased continuously, and the growth of the bacteria reached a peak at 84 hours. After that, due to the large consumption of nutrients, the products inhibiting the metabolic activity of the bacteria continued to accumulate, the growth rate of the cells decreased, and the death of the growth phase was entered. From the utilization of the nitrogen source and the carbon source, both can satisfy the growth and metabolism requirements of the bacteria throughout the fermentation process, and therefore, no intermediate

feed was required. During the fermentation process, the pH value decreases with the accumulation of secondary metabolites and other metabolites. So the results indicted a stable fermentation broth was manufactured.

Separation, Purification, and identification of compound: Fermented broth was separated by solvent extraction with the 1:1 ratio. Among four solvents (Ethyl acetate, Diethyl ether, Ethanol, Methanol, and N-Butanol) Diethyl ether had significant antifungal effects as in (Fig-2). The inhibition zone was 20 mm while all other low inhibition effects. Selected this potent extract for Silica gel column chromatography. Silica gel column chromatography purified fractions were analyzed by antifungal activity and selected the most potent for further analysis. At different concentrations of the potent fraction (0.5, 2, 4, 6, 8, 10 µg/ml) antifungal activity was varies.

Figure 1: Phylogenetic tree of *Streptomyces albidoflavus*

Figure 2: Bioassay effects of different solvent extracts against *Fusarium oxysporum*. All the inhibition zone values are mean of 3 replicates.

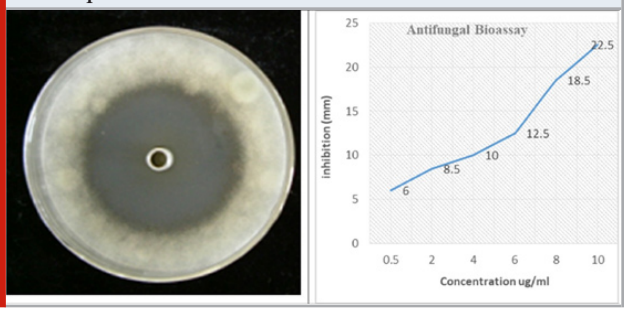


As the concentration of fraction increased from 0.5 to 10 there was increased in the activity as shown in (Fig-3). At 0.5 10 µg/ml the inhibition zone was 6mm and later on 10 10 µg/ml inhibition zone was 22.5mm. The data presented in the graph was mean of three replicates. Later on Silica gel column chromatography purified potent fraction was selected for biochemical profile identification by GC-MS technique. There were 19 compounds identified in GC-MS spectrometer profile as shown in (Fig-4). On the base of area percent, there were two compounds considered as a major composite in this extract. Morphinan, 7, 8-dihydro and Phenol, 2, 4-bis (1, 1-dimethylethyl) with the area percent of 18.71 and 18.10 respectively (Table 4).

Inhibition Effects of *S. albidoflavus* and Test Antagonistic agents against Fusarium: Among these seven agents (including *S. albidoflavus*) of biocontrol in the inhibition test, almost all of them exhibited potent inhibitory effects against *F. oxysporum*, while *Bacillus subtilis* had exhibited the most significant effect at the inhibiting rate of 94.26 %, whereas *S. albidoflavus* displayed the effects of inhibition rate of 90%. As it was observed the second most potent antagonistic agent (Table 5). From the results it indicated this novel strain have potent effects against *Fusarium oxysporum* f. sp. cubense by comparison to other test antagonists.

In this study, a soil bacterial isolate were screened against *Fusarium oxysporum* f. sp. Cubense, the causal agent of Fusarium wilt in Banana. Molecular analysis indicated the strainS128 belongs to *Streptomyces* bacteria identified as *S. albidoflavus*. It indicated from the results (Fig-1), concerned strain had potent antifungal effects against *F. oxysporum*. In previous studies indicated that, *Actinomycetes* are the major source of bioactive compounds (Hug et al. 2018). Several studies founded that *Streptomyces* can control the fungus phytopathogens likewise *Rhizoctonia solani* (tobacco target spot) (Ahsan et al. 2017), Ginseng damping-off (Van et al. 2017) and *Streptomyces plicatus* on the oomycete *Phytophthora capsici* (Chen et al. 2016). So this novel *Streptomyces* strain could be potent biocontrol antagonist against *Fusarium oxysporum* f. sp. Cubense. Recently reported that *Streptomyces* sp. AC-19 and *Bacillus* sp. BS-20 were successfully controls the Banana *Fusarium* wilt (Anusha, et al. 2019).

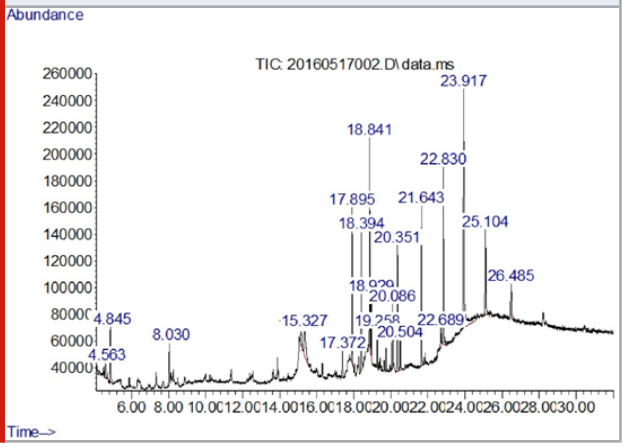
Figure 3: Antifungal bioassay of purified fraction by silica gel column chromatography against *Fusarium oxysporum* f. sp. Cubense. In the graph antifungal values are the mean of 3 replicates



For the production of active compounds in fermentation batch the best combination of factors in the fermentation medium was as followed, soluble starch 4%, peanut flour 2%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.8%, and NaCl 0.8%. The results indicated that it is necessary to make a well-balanced combination to develop the bioactive compounds. Well, balanced combination of nutrients helps out to produce maximum yield of active compounds. Optimization of nutrients not only caused in an increased of efficiency but also give the understating of nutrient

components (Gao et al. 2016). From the results, it was indicated that orthogonal design helps out to optimize the best nutrient parameter in less experiment for the production of active compounds. Experimental designs decrease the labor and cost to produce active substance from submerged fermentation. During the process of fermentation statistical designs, not only reduced the cost and work but also improved the quality and production (Elibol 2004). Solvent extracts from fermented broth showed efficacy against the pathogen. The strong activity exhibited by Diethyl ether. As diethyl ether are virtuous solvents for a comprehensive range of polar and nonpolar organic compounds (Ouellette and Rawn 2015).

Figure 4: GC-MS Profile from the extract of *Streptomyces albidoflavnous*



Potent extract of Diethyl ether from fermented broth of strain further purified by silica gel column chromatography. Several fractions assayed against the pathogen *F. oxysporum* then selected the most potent fraction. The purified fraction exhibited inhibition zone against the pathogen, which indicated from the results that the strain have the potential to control the pathogen. In earlier reports investigated that *Streptomyces* strain could control the soil borne fungus pathogens (Anusha, et al. 2019). The current study indicated that purified fractions from *S. albidoflavnous* produced 19 compounds. Production of antibiotic substances greatly depends on natural resources. In natural resources, *Streptomyces* is a major source of bioactive compounds for pharmaceutical products (Jakubiec et al. 2018).

Among 19 different compounds, there are 2 compounds of phenol were identified, one of them had a high area of percentage (18.10) Phenol, 2,4-bis(1,1-dimethylethyl) 18.0 while other had low (6.96) Phenol, 1`,1 dimethyl-ester. Phenol compounds have potent antimicrobial effects (Al-Youssef and Hassan 2015). GC-MS analysis revealed that Phenol, 2,4-bis(1,1-dimethylethyl) could be an active compound as it constitutes the major portion of this extract. This compound previously reported as antimicrobial to combat biofilm formation (Padmavathi et al. 2014). From the GC-MS analysis, there was another compound Morphinan, 7, 8-dihydro with the highest 18.71 area percentage was investigated. As previously reported that derivatives of morphine have no antimicrobial effects, but caused mammalian seizures (Jalodia et al. 2018). From the results, it indicated that S. albidoflavus had significant effects on the pathogen. In conclusion, *S. albidoflavus* is a strong antagonistic agent against Fusarium oxysporum fungus and could have broad-spectrum capability to control Fusarium wilt in banana.

CONCLUSION

This study concluded that *Streptomyces albidoflavus* identified a novel antagonistic agent against soil borne fungus pathogen *Fusarium oxysporum* f. sp. Cubense. This strain might be introduced as an effective biocontrol agent for sustainable agriculture. The results indicated that, extract from the Streptomyces strain would be a substitute to chemical substances for the disease management of Banana *Fusarium* wilt. *S. albidoflavus* strain have broad spectrum potential against fungus pathogens.

Competing Interest: There is no Competing interest.

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Authors' contributions: TA; did the experiments, write, analyze and drafting of the manuscript, WY; plan the work, supervise the work, provide funds, analyze, and proof read. All the authors have read and approved the manuscript", and ensure that this is the case.

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