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Some of Soil *Streptomyces* Isolates Decrease Toxigenic Capability of *Fusarium verticillioides in vitro*

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ABSTRACT

Fungal pathogens pose serious problems worldwide for both human and animal health and their toxin are natural contaminants of foods and feeds. *Streptomyces* had bioactive substances that demonstrated inhibitory affects against pathogenic and toxic fungi. The focus of this study was on the antimicrobial and inhibitory activities of *Streptomyces*. *Streptomyces* isolates obtained from different area in Al-Muzahimiyah in Riyadh Region at Saudi Arabia. Six different isolates were evaluated for their antimicrobial activity against different microorganisms. A laboratory studies was carried out on tested isolates of *Streptomyces* to determine their effect on toxigenic isolate of *F. verticillioides*. Out of the tested *Streptomyces* isolates, only RS-4, WS-1, GS-2 and BS-5 showed antimicrobial activity. According to antagonistic and dry weight trials it was found that all of the tested *Streptomyces* isolates had inhibitory effect on the toxigenic isolate of *F. verticillioides* especially (RS-4 followed by WS-1 and GS-2). There was strong inverse relationship between antagonism activity of tested *Streptomyces* isolates and total dry weight of indicator microorganisms. A significant reduction in zearalenone and fumonisin was recorded after 7 days of incubation. Also, *Streptomyces* isolates No. RS-4 was the most effective one followed by WS-1 and GS-2 and the reduction ratio was 65.8, 52.6 and 447%, for zearalenone while for fumonisin was 62.5, 45.8, 37.5%, respectively. In conclusion, some *Streptomyces* were isolated from ecosystem soil. These *Streptomyces* isolates were differed in their morphological and antagonistic characteristics. Some of these isolates have a significant impact in reducing mycotoxins which secreted by *F. verticillioides*.

Key words: Zearalenone, fumonisin, T-2 toxin, *Fusarium moniliforme*, detoxification

INTRODUCTION

In the last 30 years it has become clear that commonly occurring fungi growing in foods and feeds may produce mycotoxins (Pitt, 2000; Reddy *et al*., 2010). These mycotoxins may yearly contaminate about 25% of the worldwide plants crops according to the estimation of FAO organization (Fandohan *et al*., 2003; Wagacha and Muthomi, 2008).

The mycotoxin zearalenone and fumonisin are produced by several *Fusarium* species that invade corn, wheat, nuts and other cereals (Logrieco *et al*., 2002; Alwakeel and Nasser, 2011). Fumonisin and zearalenone have caused major epidemics in human and animals during historical

times. These toxins have been implicated in serious reproductive and toxicological problems to farm animals fed contaminated with mold causing hyperestrogenic syndrome and consequently a large economic loss (Matthies *et al*., 2001; Chukwuka *et al*., 2010; Iheshiulor *et al*., 2011).

The animal production performance is negatively affected by the presence of Fusarium Mycotoxins (Vekiru *et al*., 2010). The detoxification of mycotoxins in contaminated plant material was conducted by various physical, chemical and biological processes (Zinedine *et al*., 2007). The biotransformation of mycotoxins was conducted by many species of microbes to degrade these mycotoxins to nontoxic metabolites. This technology considered one of promising approaches as biotransformation of Zearalenone. The biotransformation of Zearalenone was occurred by the yeast *Trichosporon mycotoxinivoran*. The new structure of metabolite named ZOM-1. The ZOM-1 did not show estrogenic activity in a sensitive yeast bioassay. Also, ZOM-1 was safe even at a concentration 1,000-fold higher than that of ZON and did not interact with the human estrogen receptor in an *in vitro* competitive binding assay (Vekiru *et al*., 2010). The conversion of Fusarium toxins by various microorganisms was studied. A new polar metabolite was formed and the structure of the new metabolite was determined as zearalenone-4-Osulfate conjugate on the basis of enzymatic and acid hydrolysis (El-Sharkaway *et al*., 1991; Nourozian *et al*., 2006). The ability of lactic acid produced by bacteria to bind fumonisins in fermented foods and feeds and in the gastrointestinal tract could contribute to decrease their bioavailability and toxic effects on farm animals and humans (Niderkorn *et al*., 2009). The aim of the present research was to study the inhibitory effects of some *Streptomyces* isolates on the toxic secretion (Fumonisin and zearalenone) of *F. verticillioides* as promising technology.

MATERIALS AND METHODS

Sampling procedure: From 2008 to 2009, 6 farming soil samples, obtained from 20-25 cm depth, were collected in sterile containers from different area in Al-Muzahimiyah in Riyadh Region at Saudi Arabia.

Isolation of *Streptomyces* colonies from the farming soil samples: The samples were dried for 3 days at room temperature to reduce the bacterial flora which are not harmful to the growth of *Streptomyces* (Goodfellow and Minnikin, 1985). One gram of air dried soil was shaken in a flask containing 100 mL of distilled water and serial dilutions were placed on starch casein solid media (Kuster and Williams, 1964). The plates were incubated at 28±2°C until the sporulation of *Streptomyces* colonies occurred. The emerged colonies (where the mycelia remained intact and the aerial mycelia and long spore chain were abundant) were picked up and transferred to a starch nitrate medium (Lechevalier and Lechevalier, 1970). Pure cultures were obtained from selected colonies for repeated sub-culturing.

Morphological characterization: *Streptomyces* colonies on glycerol-nitrate-casein agar were transferred onto oatmeal agar, streaked across the plate and incubated in the dark at 27°C for 21 days.

Color determination: An agar disk of a grown *Streptomyces* culture was removed aseptically and placed on a seeded plate of the tested organisms. Observation was mad after 21 days for: a) Mass color or mature, sporulating aerial surface growth, b) The color of substrate mycelium as viewed from the reverse side and c) Diffusible soluble pigments other than melanin. Observation was made

after 21 days of incubation and was limited to mature cultures with heavy spore mass surface using code for determining the color of aerial mycelium of Streptomycetes composed by Prauser for color tabs of Baumann Farbtonkarte Atlas I (Merriman *et al.*, 1974).

Morphological characteristics of the spore bearing hyphae: The spore-bearing hyphae characteristics were determined by direct microscopic examination of the culture surface (21 days old) on opened dishes of the crosshatched cultures using 100×magnifications. The species involved in the genus *Streptomyces* divide into sections: Rectus (R) or straight, Flexible (F) or flexuous, Retinaculum-Apertum (RA) and Spiral (S) (Nonomura, 1974).

Antagonistic characterization

Submerged culture: Preliminary screening for antibiotic production was done by conventional spot inoculation method on agar medium. Subsequent screening of promising isolates was done using submerged culture. Pure streptomycetes isolates were spot inoculated on actinomycetes isolation agar medium (Difco, NJ, USA) (Locci, 1989). The culture broth was separated from the mycelium by centrifugation at 5000 rpm for 10 min. The supernatant was sterilized by filtration and used for the evaluation of the inhibitory activity by agar well diffusion method against test microorganism as *Escherichia coli, Bacillus. cereus, S. aureus, F. monilliorme, S. cerevisiae, C. albicans, P. aeruginosa* and *A. niger* (Romero *et al.*, 1984).

Well diffusion-method: For evaluation of antibacterial activity of aqueous samples, by use of sterile cork borer, wells (6×4 mm, 2 cm apart) were punctured in fresh bacterial lawn-cultures. Plates were incubated at 29°C for 24 h. Bioactivity was determined by measuring inhibitory zones (mm). Each experiment was repeated three times and the mean of inhibitory zones recorded. Controls included use of blank wells of sterile distilled water (Dhingra and Sinclair, 1995).

Total dry weight (TDW): Loopful of the tested *Streptomyces* and disc (1 mm) of *F. verticillioides* (1 mm) were inoculated in minimal volume of media containing 3 gm Potassium dihydrogen phosphate, 6 g Di-sodium orthophosphate, 5 g NaCl 2 g NH_4Cl, 0.1 g $MgSO_4$, 8 g Glucose 1 L distilled water. These flasks were incubated at 28°C for 7 days at 120 rpm. The dry cell weight was determined as follows: mycelia were harvested by centrifugation at 8000 g for 10 min and 4°C. After separation, the cells were re-suspended in distilled water, washed twice with distilled water and dried at 105°C for 24 h.

Classification of *Streptomyces* strain: The isolated organisms were characterized morphologically. The majority of the methods were used as had been described in Bergey's manual of systematic bacteriology (Williams *et al.*, 1989). *Streptomyces* colonies were characterized morphologically and physiologically following the direction mentioned in the methods manual of international cooperative project for description and deposition of cultures of *Streptomyces* (ISP), (Shirling and Gottlieb, 1966).

Zearalenone and fumonisin bio-degradation ratio: The most active isolates of *Streptomyces* (WS-1, GS-2, RS-4 and BS-5) were chose to conduct this experiment especially these isolates had largest inhibition zone with tested isolate of *Fusarium verticillioides*. The supernatant of former experiment was used to detect fumonisin, Zearalenone and the biodegradation materials. The *Streptomyces* isolates were examined against reference material of *Fusarium* mycotoxins

individually in flasks as: Loopful of *Streptomyces* culture were inoculated in minimal media (as mention before) with 1 μg mL^{-1} of ultrapure Zearalenone and fumonisin. These flasks were incubated at 28°C for 7 days at 120 rpm and then filtrate through micro filter 45 μm in control treatment the reference material was replaced with fumonisin toxins were determined according to the method described by Mazzani *et al.* (2001). The Zearalenone concentration was determined as mentioned in the fumonisin toxin but the dilution was made with 49 mL distilled water which were passed through Zearatest column (Vicam company) and then measured in calibrated fluorometer model (Series-4/Vicam) according to the method of Martins *et al.* (2003).

Statistical analysis: The obtained data were statistically analyzed using the Analysis of Variance (ANOVA) one way with the MSTAT-C statistical package The Least Significant Difference procedure (LSD) was used at 0.05 level of probability (Fisher, 1948).

RESULTS

Morphological characterizations: The *Streptomyces* isolates was isolated from AL-Muzahimiyah in Riyadh region and distribution, occurrence and characteristics was observed in addition to the aerial hyphae bears spores with different shapes spiral, flexuous, rectus and retinaculum apertum (Table 1). Also, the number of these spores differed between tested *Streptomyces* isolates. The isolate WS-1 and YS-3 showed different spore's shapes followed by GS-2, GS-6, RS-4 and BS-5, respectively. The flexuous was occurred in all isolates except isolates No. BS-5.

The results in Table 2 indicated that the aerial mass color varied between white, gray, yellow, red, blue and green with the exception of neutral agar medium. The substrate mycelium color varied depending on the medium composition.

The isolates No. WS-1, GS-2 and YS-3 had ability to excrete melanin, soluble pigment and distinctive reverse side pigment while the GS-6 isolate formed only the soluble pigment and

Table 1: Morphological characterizations of different *Streptomyces* isolates

	Spore bearing hyphae					
Streptomyces	WS-1	GS-2	YS-3	RS-4	BS-5	GS-6
Spiral	27^{b}	44^{a}	11^{a}	0^{b}	0^{b}	0^{c}
Flexous	12^{c}	31^{b}	44^{b}	50^{a}	0^{b}	43^{b}
Rectus	27^{b}	0^{d}	33^{d}	50^{a}	0^{b}	57^{a}
Retinaculum apertum	35^{a}	25^{c}	11^{c}	0^{b}	100^{a}	0^{c}
LSD at 5%	4.354	2.378	5.201	5.612	1.105	4.517

Values in column with different letter are significant different

Table 2: Color characteristics and percentage of pigments production for tested *Streptomyces* isolates

	Pigments production for tested isolates (%)					
Tested characteristics	WS-1	GS-2	YS-3	RS-4	BS-5	GS-6
Melanin	10^{c}	40^{a}	33^{b}	0^{b}	100^{a}	0^{c}
Soluble pigment	35^{a}	4^{b}	66^{a}	0^{b}	0^{b}	80^{a}
Distinctive reverse side pigment	25^{b}	8^{b}	33^{b}	100^{a}	0^{b}	20^{b}
LSD at 5%	3.926	3.460	5.704	0.926	0.755	4.596

Values in column with different letter are significant different

distinctive reverse side pigment. On the other hand both isolates RS-4 and BS-5 failed to excrete anyone except distinctive reverse side pigment and melanin, respectively. There are variations in the percentage of pigments production between tested isolates. The high percentage of melanin was recorded with BS-5 while the most important (major) producer for soluble pigment was GS-6 isolate. The RS-4 produced the highest concentration of distinctive reverse side pigment.

Antagonistic activities: The antimicrobial activities for different *Streptomyces* tested isolates against some fungi and bacteria indicators were recorded in Table 3. There was not any activity or inhibition zone recorded by isolate No. YS-3 and GS-6 while the highest antagonism effect was recorded with RS-4 followed by isolate WS-1, BS-5 and GS-2, respectively. The diameter of inhibition zone for RS-4 isolate against tested isolate of *F. verticillioides* was 17 mm followed by WS-1, GS-2 and BS-5 as 11, 7 and 5 mm, respectively. The main trend of tested isolates of *Streptomyces* was recorded against *F. verticillioides* by equation Y = 0.0536 X+1.7507 (Fig. 1). This trend was ascending and progressive with diameter of inhibition zone.

Total dry weight: The obtained result in Table 4 show, that the examined isolates of *Streptomyces* had great effect to reduce the dry weight for all microorganisms indicators comparing with control. The most reduction of dry weight for *F. verticillioides* recorded with GS-2, RS-4, WS-1, GS-6 and BS-5 isolate being 1.20, 1.32, 1.35, 2.25, 2.34 and 2.53 g, respectively comparing with control 2.45 g. Isolate YS-3 had different trend and the dry weigh was higher than that of control 2.53 g. The main trend of dry weight (Fig. 1) was descending and displayed with equation:

$$Y=-0.0714X+6$$

Table 3: Antimicrobial activities of different *Streptomyces* isolates against tested microorganisms

	Diameter zone of inhibition (mm)						
Indicators[a]	Control	WS-1	GS-2	YS-3	RS-4	BS-5	GS-6
Aspergillus niger	0	7^{c}	3^{f}	0	14^{d}	9^{a}	0
Fusarium verticillioides	0	11^{a}	7^{e}	0	17^{c}	5^{b}	0
Pseudomonas aeruginosa	0	9^{ab}	13^{b}	0	18^{c}	3^{b}	0
Bacillus cereus	0	4^{d}	11^{c}	0	27^{a}	8^{a}	0
Escherichia coli	0	9^{b}	9^{d}	0	22^{b}	3^{b}	0
*S*taphylococcus *aureus*	0	5^{d}	16^{a}	0	19^{c}	5^{b}	0
LSD at 5%	NS	1.683	1.473	NS	2.020	2.965	NS

Values in column with different letter are significent different

Table 4: Effect of different tested *Streptomyces* isolates on dry weight for tested indicators

	Total dry weigh (g)						
Tested indicators	Control	WS-1	GS-2	YS-3	RS-4	BS-5	GS-6
Aspergillus niger	2.58^{a}	1.65^{a}	1.86^{a}	2.61^{a}	2.52^{a}	1.27^{b}	2.37^{a}
F. verticillioides	2.45^{a}	1.35^{a}	1.20^{b}	2.53^{b}	1.32^{b}	2.34^{a}	2.25^{b}
Pseudomonas aeruginosa	0.71^{b}	0.50^{b}	0.41^{c}	0.65^{d}	0.65^{c}	0.68^{d}	0.65^{cd}
Bacillus cereus	0.65^{b}	0.42^{b}	0.34^{c}	0.59^{e}	0.39^{c}	0.62^{e}	0.60^{d}
E. coli	0.79^{b}	0.53^{b}	0.44^{c}	0.72^{c}	0.72^{c}	0.74^{c}	0.72^{c}
*S*taphylococcus *aureus*	0.43^{c}	0.38^{b}	0.31^{c}	0.39^{f}	0.39^{c}	0.40^{f}	0.39^{e}
LSD at 5%	0.173	0.539	0.257	0.058	0.578	0.058	0.814

Values in column with different letter are significant different

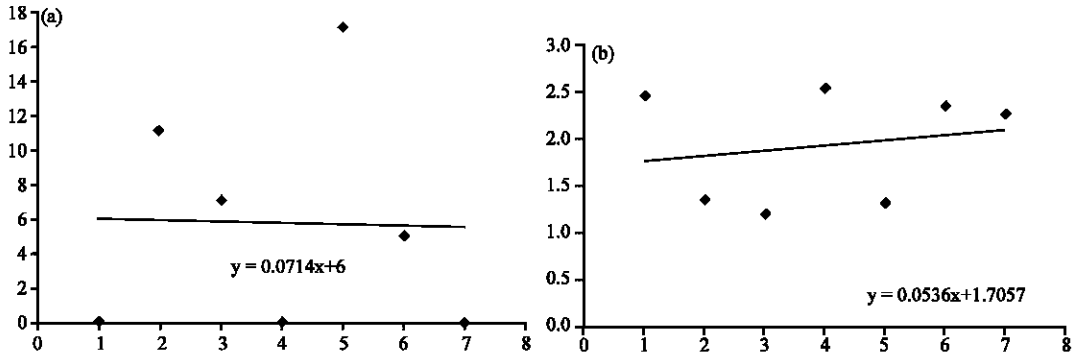

Fig. 1 (a-b): The chromatogram of main trend line equation for *Streptomyces* isolates; (1) control, (2) WS-1 (3) GS-2 (4) YS-3 (5) RS-4 (6) BS-5 and (7) GS-6 for both inhibition zone and dry weight against tested isolates of *F. verticillioides* (a) Antagonism (mm) and (b) Dry weight (g)

Table 5: Effect of different tested *Streptomyces* isolates on fumonisin and zearalenone biodegradation

	Mycotoxins concentration (μg mL^{-1})			
	Reference material		*Fusarium verticillioides*	
Streptomyces isolates	Fumonisin	Zearalenone	Fumonisin	Zearalenone
WS-1	0.31^{b}	0.43^{bc}	1.0^{bc}	1.7^{c}
GS-2	0.37^{b}	0.49^{b}	1.2^{b}	2.0^{b}
RS-4	0.29^{b}	0.35^{c}	0.7^{c}	1.1^{d}
BS-5	0.30^{b}	0.37^{c}	0.9^{c}	1.2^{d}
Control	1.00^{a}	1.00^{a}	2.4^{a}	3.8^{a}
LSD at 5%	0.089	0.011	0.363	0.310

Values in column with different letter are significant different

Taxonomy: The active strain of *Streptomyces* (WS-1, GS-2, RS-4 and BS-5) were identified as *Streptomyces plicatus, St. rimosus, St. rutgersensus* and *St. griseus*, respectively based on complete morphological characterization criteria for this isolates as well as biochemical properties, BIOLOGE analysis and the literature reviews of classification key but the confirmation with DNA sequence was not be available.

Inhibition and biodegradation of mycotoxins: The data in Table 5 indicate that the tested isolates of *Streptomyces* had version ability to reduce the concentration of Fusarium mycotoxins i.e., fumonisins and zearalenone in reference material and also with *Fusarium verticillioides* supernatant culture. The biodegradation of fumonisins was more than zearalenone with all tested *Streptomyces* isolates. Isolate RS-4 was the most effective one to biodegraded fumonisins toxins followed by WS-1 and GS-2 in media contains reference material. In *Fusarium verticillioides* culture; the fumonisins production was reduced more than zearalenone by tested isolates of *Streptomyces*. Also *Streptomyces* isolates RS-4 was the most effective followed by WS-1 and GS-2 and reduction ratio was 65.8,52.6 and 447%, respectively for zearalenone while for fumonisins was 62.5, 45.8,37.5%, respectively.

DISCUSSION

The characterization of *Streptomyces* species is mainly based on the substrate mycelia color, soluble pigment production. The abundance and the color of aerial mycelium depended on the medium composition and the age of the culture. The tested isolates of *Streptomyces* recorded differences in color and spore shape. The morphological examination of *Streptomyces* isolates which were active on the test organisms, indicates that these belong to the *Streptomyces* genus and all of these results were in parallel with Waksman (1961), Shirling and Gottlieb (1966), Nonomura (1974), Williams *et al.* (1983), Cross (1989), Goodfellow and Lechevalier (1989) and Locci (1989). There is no any correlation relationship between morphology and the antagonism of these isolates.

There was strong inverse relationship between antagonism activity for tested isolates of *Streptomyces* and total dry weight for indicator microorganisms. It was found that all of the *Streptomyces* isolates had an inhibitory effect on the toxigenic *F. verticillioides* especially isolates (RS-4 followed by WS-1, BS-5 and GS-2, respectively) by reducing zearalenone and fumonisin content after 7 days of incubation. This may be due to antibiosis whereas the biocontrol agent (*Streptomyces*) produce one or more substance that inhibits or kill the pathogen and these results were in harmony with Chamberlain and Crawford (1999), Crawford *et al.* (1993) and Trejo-Estrada *et al.* (1998). Reducing of fusarium mycotoxins may be due to bioactive compounds excreted from *Streptomyces*. These compounds showed anti-fungal activity such as 4' phenyl-1-naphthyl-phenyl acetamide as mentioned by Atta and Ahmad (2009). The competition between *Streptomyces* and toxigenic fusarium on nutrition, inorganic compounds and space may be one of the mechanisms explain the *Streptomyces* antagonism and mycotoxins detoxification. *Streptomyces* may be utilized the mycotoxins and another specific compounds produced by fusarium in their energy pathway. Iron can play a role in growth inhibition of Fusarium and well-known source of competition between fusarium and *Streptomyces* (Dowling and O'Gara, 1994). *Streptomyces* produce iron-chelating compounds, siderophores that starve pathogen for iron (Tokala *et al.*, 2002). Parasitism, mycoparasitism of fungal pathogens can sometimes be attributed to the production of lytic enzymes such as Chitinase and **β**-1, 3 glucanase (Kavitha *et al.*, 2010; Gonzalez-Franco *et al.*, 2003; Berg *et al.*, 2002; Valois *et al.*, 1996) which play important role in the hydrolysis of chitin and glucan of the fungal cell wall. These hydrolases initiate the process of physical destruction of the Fungal Cell Walls (FCW) (Adams, 1990).

The fungal growth may be affecting with antimicrobial material produced by *Streptomyces* as well as due to competition for space or food or oxygen in the vicinity of growth environment and this may affect physiological and metabolic processes.

According to antagonisitic and dry weight trials, it was found that all of the *Streptomyces* isolates had an inhibitory effect on the toxigenic *F. verticillioides* especially isolates (RS-4 followed by WS-1, BS-5 and GS-2, respectively) by reducing zearalenone and fumonisin content after 7 days of incubation.

CONCLUSION

Many of the *Streptomyces* isolates were isolated from Al-Muzahimiyah ecosystem soil in Riyadh Region at Saudi Arabia. These *Streptomyces* were isolated from ecosystem soil. These *Streptomyces* isolates were differed in their spore shape, physiological morphological and antagonistic characteristics. Four of these isolates had a significant impact in reducing mycotoxins which secreted by *F. verticillioides* although, there was a variation in efficiency of these isolates. These isolates can be used to reduce the level of mycotoxin contamination, especially in the feed industry.

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