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RESEARCH ARTICLE

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Optimization of culture conditions and antimicrobial activity of *Streptomyces longisporoflavus*

ABSTRACT:

In the present study *Streptomyces longisporoflavus* was screened for antimicrobial activity against fourteen multi-drug resistant pathogenic bacteria (*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella pneumonia Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes*), and fungi (*Trichosporon mucoides, Candida albicans, Candida glabrata, Candida krusie, Candida dubliniensis and Candida tropicalis*) by using well- agar diffusion method. *S. longisporoflavus* showed high antimicrobial activity only against seven of the fourteen tested MDR bacteria and fungi (*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes* and *Candida tropicalis).* Cultural conditions of *S. longisporoflavus* were optimized for maximizing growth and antimicrobial activity. The results under the optimized conditions recorded the maximum mycelial dry weight and significant antimicrobial activity against all the seven MDR pathogenic bacteria, with incubation period 8 days, at pH 7.0, temperature 55°C, media supplemented with potassium nitrate as nitrogen and starch as carbon sources. The highest diameters of inhibition zones by the antimicrobial materials were recorded with MDR *Staph. aureus* followed by *E. coli* and *proteus mirabilis* (37, 35, and 34 mm, respectively), at all optimal cultural conditions of *S. longisporoflavus*.

KEY WORDS:

Streptomyces longisporoflavus, optimization, antimicrobial activity, MDR pathogenic bacteria and fungi.

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INTRODUCTION:

The search for bioactive metabolites including novel antibiotic compounds from microbial sources for potential use in agricultural, pharmaceutical, and industrial applications has become more important due to the development of drug/multi-drug resistance in most of the pathogenic microbes. Researchers across the globe are aggressively searching for new, potent, sustainable, and broad- spectrum antimicrobial compounds from various sources including microbes (Hayakawa, 2008). Actinomycetes are diverse group of Gram positive bacteria that usually grow by filament formation. They belong to the order Actinomycetales (Super kingdom: Bacteria, Phylum: Firmicutes, Class: Actinobacteria, Subclass: Actinobacteridae) (Ventura *et al*., 2007). Actinobacteria are widely distributed in both terrestrial and aquatic ecosystems, mainly in soil, where they play an essential role in recycling refractory biomaterials by decomposing complex mixtures of polymers in dead plants animals and fungal materials (Naikpatil and Rathod, 2011). Since hot spring environment differs greatly from terrestrial habitats, the biological characteristics of actinomycetes and their distribution are expected to be different from those of soil actinomycetes, Furthermore, the hot spring

environment is proving to be a major source of new natural products, especially antimicrobial compounds, most notably those expressed by actinomycetes (Bull *et al*., 2005). Antibiotics and other bioactive compounds from actinomycetes are renowned for their antibacterial, antifungal, neuritogenic, anticancer, antialgal, antimalarial, and anti-inflammatory activities (Saxena *et al*., 2013). Rakshanya *et al*. (2011) isolated actinomycetes and the isolates were identified as *Streptomyces* sp., *Micromonospora* sp., and *Nocardia* sp., these selected isolates were able to inhibit the growth of different microorganisms (*S. aureus, Proteus vulgaris, P. aeruginosa, E. coli, B. subtilis, B. megaterium, K. pneumoniae, C. albicans, Aspergillus niger,* and *S. cervisiae*). Out of 5 actinomycete isolates, *Streptomyces* sp. exhibited high antibacterial activity against *Staphylococcus aureus*. Production of secondary metabolites by microorganisms differs qualitatively and quantitatively depending on the strains and species of microorganisms used, as well as of their nutritional and cultural conditions (Valli *et al*., 2012). Parameters like initial pH, temperature, etc., have a profound effect on the production of bioactive metabolites. The nutritional sources of carbon and nitrogen are known to have a profound effect on the antibiotic production by actinomycetes (Himabindu and Jetty, 2006) Previously, Bundale *et al*. (2015) reported that monosaccharides are suitable sources for the growth of actinomycetes. Jonsbu *et al*. (2002) reported that due to species specific variation, different *Streptomyces* species require different types of carbon sources for cell growth and secondary metabolite production. Al-Zahrani (2007) reported that maximum antibacterial agents biosynthesis was obtained in medium supplemented with starch as a sole carbon source followed by glucose. Sujatha *et al*. (2005) found that maximum antibiotic production was obtained in medium supplemented with glucose as a sole carbon source followed by fructose and glycerol. The highest antibiotic production was obtained in culture of isolate BT-408 containing ammonium nitrate as a nitrogen source, followed by cultures containing sodium nitrate, potassium nitrate and alanine, BT-408 showed a narrow range of incubation temperature for relatively good growth and antibiotic production. Wang *et al*. (2010) reported that the optimal primary pH was around 7.0 for production of compounds with antimicrobial activity by actinomycete strain Hhs.015T. The present study aimed to improve the inhibitory effect of the antimicrobial materials produced by *S. longisporoflavus* by optimizing its physical and chemical parameters and study its antimicrobial activity against the tested MDR pathogenic bacteria and fungi.

MATERIAL AND METHODS:

Producing organism:

Streptomyces longisporoflavus was kindly provided by microbiology unit, School of the environment and Safety Engineering, Jiangsu University, China, then it was maintained using dense spores from 2 plates of *S. longisporoflavus* and mixed with about 5 ml of 10 % (v/v) sterilized glycerol with sterilized glass bead (diameter, 0.45 cm) separately. The suspension of *S. longisporoflavus* was shacked thoroughly, and then stored at -20°C for further work.

Media used:

The composition of the used media in the present study was in g/l. All components of each medium were weighted and dissolved in 1000 ml of distilled water by stirring. The pH was adjusted to pH 7.0 and the media was autoclaved at 121°C for 20 min.

The media compositions were as follows: Starch-nitrate agar medium consist of :20.0 g Starch, 1.0 g KH_2PO_4, 2.0 g KNO_3, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7\ H_2O$, 3.0 g $CaCO_4$, 0.5 g NaCl, and 20.0 g agar. One ml of the trace salt solution was added. Trace salt solution consist of 0.1 g $FeSO_4.7\ H_2O$, 0.1 g $MnGl_2.4H_2O$, 0.1 g $ZnSO_4.7\ H_2O$, and 100.0 ml distilled water (Shirling and Gottlieb, 1966). Nutrient agar medium consists of: 5.0 g Peptone, 3.0 g Meat extract (Beef extract), 5.0 g NaCl and 20.0 g agar (Difco, 1953). Sabouraud medium consist of 20.0 g Glucose, 10.0 g peptone, and 20.0 g agar (Booth, 1971).

Tested MDR bacteria and fungi:

Antimicrobial activity of *S. longisporoflavus* was tested against eight species of MDR bacteria namely *E. coli, Proteus mirabilis, P. aeruginosa, Salmonella typhimurium, K. pneumonia, Staph. aureus, B. cereus, L. monocytogenes),* and six species of MDR fungi (*Tricosporon mucoides, Candida albicans, Candida glabrata, Candida krusie, Candida dublinesis and Candida tropicalis*). These MDR pathogenic bacteria and fungi were obtained from Tanta University Hospitals.

Screening of *S. longisporoflavus* for its antimicrobial:

One ml of spore suspension of *S. longisporoflavus* was cultured in 250 Erlenmeyer flasks, each containing 50 ml of autoclaved starch-nitrate medium. Three replicates were used. After incubation of culture flasks on a rotary shaker at 45°C for 7 days, the culture of *S. longisporoflavus* was centrifuged at 3000 rpm for 30 min and the supernatant was collected for determination of its antimicrobial activity, 1 ml of (10^7 CFU/ ml) old broth cultures 18hrs of the eight MDR tested bacterial and 1 ml of (10^6 cell/ml) 72hrs broth cultures of the

six MDR fungal strains were swabbed separately on freshly prepared nutrient agar and sabaroud's dextrose agar plates, respectively. Wells 5 mm in diameter were made on the inoculated plates separately using cork borer and each well loaded with 100 µl of mycelial free culture supernatant of *S. longisporoflavus*. The plates containing the MDR pathogenic bacterial species were incubated at 37°C for 24 hrs and the plates containing the MDR pathogenic fungal species were incubated at 28°C for 4-5 days. After incubation time, the diameters of inhibition zone of the tested bacteria and fungi were measured (Holmalahti *et al*., 1994).

Optimization of cultural conditions of *S. longisporoflavus*:

Effect of different incubation periods on growth and antimicrobial activity of *S. longisporoflavus*:

The effect of different incubation period was carried out using 250 Erlenmeyer flasks, each containing 50 ml starch-nitrate medium, after autoclaving, each flask was inoculated separately with 100 µl (10^6 spore/ml) of spore suspension of *S. longisporoflavus.* The inoculated flasks were incubated at 45°C in a rotary shaker (120 rpm) at different incubation periods (2, 4, 6, 8, and 10 days). Three replicates were used for each incubation period. After each incubation period, each culture was centrifuged at 3000 rpm for 30 min, the supernatant at each period was taken to evaluate the antimicrobial activity of *S. longisporoflavus* on the selected MDR pathogenic bacteria and fungi (*E. coli, Proteus mirabilis, P. aeruginosa, Staph. aureus, B. cereus, L. monocytogenes* and *C. tropicalis*) by using well diffusion method as described before. The biomass (the mycelia) was dried for 24 hrs at 50°C and the dry weight (g/50 ml) was determined as described by Singh *et al*. (2014).

Effect of different pH values on growth and antimicrobial activity of *S. longisporoflavus*:

Flasks containing starch-nitrate medium were adjusted at different pH values (3, 4, 5, 7, and 9). Three replicates were used for each pH value. After autoclaving, the flasks were inoculated separately with 100 µl (10^6 spore/ml) of *S. longisporoflavus* and incubated at 45°C for the optimum incubation period (8 days). After incubation, the supernatant was prepared and tested for antimicrobial activity against the selected pathogenic bacteria and fungi using well diffusion method as mentioned before. The mycelial dry weight was determined for each pH value.

Effect of different incubation temperatures on growth and antimicrobial activity of *S. longisporoflavus*:

Flasks containing starch-nitrate medium were adjusted at the optimum pH (7.0), inoculated separately with 100 µl (10^6 spore/ml) of *S. longisporoflavus* and incubated at different temperatures (45, 50, 55, 60, and 65°C). Three replicates were used for each temperature. After incubation, the supernatant was prepared and tested for its antimicrobial activity against the selected MDR pathogenic bacteria and fungi using well diffusion method as mentioned before. The mycelial dry weight was determined for each incubation temperature.

Effect of different nitrogen sources on growth and antimicrobial activity of *S. longisporoflavus*:

Different nitrogen sources as potassium nitrate, ammonium sulphate, sodium nitrate, peptone, calcium nitrate, and tryptone were added equimolecular weight separately (0.2% w/v) to flasks containing starch-nitrate medium without any nitrogen source and was adjusted at the optimum pH 7, inoculated with 100 µl (10^6 spore/ml) of *S. longisporoflavus* and incubated at the optimum temperature 55°C for 8 days. Three replicates were used for each nitrogen source. After incubation, the supernatant was collected and tested for antimicrobial activity against the selected MDR pathogenic bacteria and fungi using well diffusion method as mentioned before. The mycelial dry weight was determined for each nitrogen source

Effect of different carbon sources on growth and antimicrobial activity of *S. longisporoflavus*:

Starch in starch-nitrate medium was replaced with 2% (w/v) of glucose, fructose, maltose, lactose, and arabinose, the culture media was adjusted at pH 7.0, autoclaved, and inoculated separately with 100 µl (10^6 spore/ml) of *S. longisporoflavus* and incubated at 55°C for 8 days. Three replicates were used for each carbon source. After incubation, the supernatant was prepared and tested for its antimicrobial activity against the selected MDR pathogenic bacteria and fungi using well diffusion method as mentioned before. The mycelial dry weight was determined for each carbon source.

Statistical analysis:

Data were statistically analysed, using analysis of variance ANOVA, (one and two-way classification).

RESULTS:

Screening of *S. longisporoflavus* for its antimicrobial:

The results in table 1 showed that out of the fourteen tested MDR pathogenic bacterial and fungal species, seven MDR species showed zones of inhibition by the antimicrobial activities of *S. longisporoflavus*. Among the seven selected MDR pathogenic species, *Staph. aureus, E. coli, and Proteus mirabilis* recorded the highest diameters of

inhibition zones which were 33, 32, and 30 mm, respectively. No inhibitory effect was recorded with *K. Pneumoniae, Salmonella typhimurium, T. mucoides, Candida glabrata, C. krusei, C. albicans* and *C. dubliniensis*.

Table. 1. Screening of *S. longisporoflavus* for its antimicrobial activity

MDR Pathogenic bacterial and fungal species	Inhibition zone diameters (mm) *S. longisporoflavus*
Escherichia coli	32 ± 1.0
Proteus mirabilis	30 ± 1.0
Listeria monocytogenes	12 ± 0.6
Bacillus cereus	17 ± 1.0
Staphylococcus aureus	33 ± 1.0
Pseudomonas aeruginosa	16 ± 0.6
Klebsiella Pneumoniae	0.0 ± 0.0
Salmonella typhimurium	0.0 ± 0.0
Candida tropicalis	15 ± 1.0
Tricosporon mucoides,	0.0 ± 0.0
Candida glabrata	0.0 ± 0.0
Candida krusei	0.0 ± 0.0
Candida albicans	0.0 ± 0.0
Candida dubliniensis	0.0 ± 0.0

±: Standard error of means

Optimization of cultural conditions of *S. longisporoflavus*:

Effect of different incubation periods on growth and antimicrobial activity of *S. longisporoflavus*:

The effect of incubation periods (2 - 10 days) on the dry weight of *S. longisporoflavus* and antimicrobial activity were studied. The results in table 2 showed that the mycelial dry weight of *S. longisporoflavus* and diameters of inhibition zones of all selected MDR bacterial and fungal species were increased continuously from the 4th to the 8th day, where the maximum dry weight and diameters of inhibition zones were obtained. Further increasing in incubation times showed gradual decrease in the dry weight of *S. longisporoflavus* and the diameters of inhibition zones with all MDR selected pathogenic species. The highest inhibitory effect of antimicrobial activity of *S. longisporoflavus* was recorded with *staph. aureus, E. coli, and P. mirabilis* at the 8th day of incubation where the diameters of inhibition zones were 35, 34, and 32 mm, respectively. Therefore, optimum incubation period to improve the production of antimicrobial material was at 8 days.

Table. 2. Effect of different incubation periods on growth and antimicrobial activity of *S. longisporoflavus*

Incubation periods	2 days	4 days	6 days	8 days	10 days	F value	P value
S. longisporoflavus dry weight (g/50 ml)	0.001	0.012	0.039	0.053	0.053	1191	0.0000***
MDR Pathogenic bacterial and fungal species	Inhibition zones diameter (mm)						
E. coli	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	25 ± 1.0^{b}	34 ± 1.0^{a}	22 ± 1.0^{c}	1191	0.0000***
Proteus mirabilis	0.0 ± 0.0^{d}	18 ± 0.6^{c}	20 ± 1.0^{b}	32 ± 1.0^{a}	17 ± 1.0^{c}	589.15	0.0000***
L. monocytogenes	0.0 ± 0.0^{c}	13 ± 1.0^{b}	15 ± 0.6^{a}	15 ± 1.0^{a}	0.0 ± 0.0^{c}	407	0.0000***
Bacillus cereus	0.0 ± 0.0^{d}	9 ± 0.6^{c}	11 ± 1.0^{b}	20 ± 1^{a}	9 ± 1.0^{c}	227	0.0000***
Staph. aureus	0.0 ± 0.0^{e}	9 ± 0.6^{d}	13 ± 1.0^{c}	35 ± 0.6^{a}	15 ± 1.0^{b}	961.8	0.0000***
Pseudomonas aeruginosa	0.0 ± 0.0^{c}	11 ± 0.6^{b}	15 ± 1.2^{a}	17 ± 1.0^{a}	11 ± 0.6^{b}	224	0.0000***
Candida tropicalis	0.0 ± 0.0^{d}	13 ± 0.6^{c}	18 ± 1.0^{b}	21 ± 1.2^{a}	14 ± 1.0^{c}	270.06	0.0000***

± Standard error of means

Values with the same letter in the same row are non-significant at $p \leq 0.05$

Effect of different pH values on growth and antimicrobial activity of *S. longisporoflavus*:

The effect of different pH values (3, 4, 5, 7, and 9) on the growth and antimicrobial activity of *S. longisporoflavus* were studied. The results in table 3 revealed that an initial pH 7.0 was the optimal for mycelial dry weight of *S. longisporoflavus* (0.053 g/50 ml) and its highest antimicrobial activity against all the selected MDR bacteria and fungi. Above and below pH 7.0 (alkaline and acidic pH, respectively), the mycelial dry weight of *S. longisporoflavus* and the diameters of inhibition zones were substantially lower with all the selected MDR bacteria and fungi. The highest inhibitory effect of antimicrobial material of *S. longisporoflavus* was recorded with *staph. aureus* (34 mm), followed by *E. coli, and P. mirabilis* where the diameters of inhibition zones were 34 and 32 mm, respectively.

Table 3. Effect of different pH values on growth and antimicrobial activity of *S. longisporoflavus*

pH values	3	4	5	7	9	F value	P value
S. longisporoflavus dry weight (g/50 ml)	0.008	0.019	0.023	0.053	0.029	387.8	0.0000***
MDR Pathogenic bacterial and fungal Species	Inhibition zones diameter (mm)						
E. coli	0.0 ± 0.0^{d}	9 ± 0.6^{c}	10 ± 1.0^{c}	34 ± 1.0^{a}	12 ± 1.0^{b}	712	0.0000***
Proteus mirabilis	0.0 ± 0.0^{d}	9 ± 1.0^{c}	13 ± 1.0^{b}	32 ± 1.0^{a}	0.0 ± 0.0^{d}	863	0.0000***
L. monocytogenes	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	10 ± 1.0^{c}	15 ± 0.6^{a}	14 ± 1.0^{b}	356.2	0.0000***
Bacillus cereus	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	10 ± 1.0^{c}	20 ± 1.0^{a}	13 ± 1.0^{b}	374	0.0000***
Staph. aureus	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	11 ± 1.0^{b}	35 ± 0.6^{a}	10 ± 0.6^{b}	1191.3	0.0000***
Pseudomonas aeruginosa	0.0 ± 0.0^{d}	10 ± 0.6^{c}	13 ± 1.0^{b}	17 ± 0.6^{a}	12 ± 0.6^{b}	322	0.0000***
Candida tropicalis	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	15 ± 0.6^{b}	21 ± 0.6^{a}	15 ± 1.0^{b}	882.3	0.0000***

± Standard error of means

Values with the same letter in the same row are non-significant at p ≤ 0.05

Effect of different incubation temperatures on growth and antimicrobial activity of *S. longisporoflavus*:

Production of antimicrobial materials by actinomycetes and the growth rate highly depended on the temperature. The results presented in table 4 showed that the mycelial dry weight of *S. longisporoflavus* and its antimicrobial activity against all the selected MDR bacterial and fungal species increased with increasing temperature up to 55°C where at which the maximum dry weight of *S. longisporoflavus* and the diameters of inhibition zones of all the selected MDR bacterial and fungal species were recorded. However higher temperatures at 60 and 65°C showed adverse effect on both mycelial dry weight and antimicrobial activity of *S. longisporoflavus* against all the selected MDR bacterial and fungal species. The results also revealed that *Staph. aureus*, *E. coli, and P. mirabilis* were the most selected MDR bacterial strains inhibited by the antimicrobial materials of *S. longisporoflavus* where the diameters of inhibition zones were 37, 35 and 34 mm, respectively.

Table 4. Effect of different incubation temperatures on growth and antimicrobial activity of *S. longisporoflavus*

Incubation temperatures (°C)	45°C	50°C	55°C	60 °C	65°C	F value	P value
S. longisporoflavus dry weight (g/50 ml)	0.022	0.027	0.055	0.015	0.007	711.4	0.0000***
Pathogenic bacterial and fungal species	Inhibition zones diameter (mm)						
E. coli	34 ± 1.0^{a}	34 ± 1.0^{a}	35 ± 1.0^{a}	11 ± 0.6^{b}	0.0 ± 0.0^{c}	1199.5	0.0000***
Proteus mirabilis	32 ± 1.0^{a}	33 ± 1.0^{a}	34 ± 0.6^{a}	10 ± 1.0^{b}	10 ± 1.0^{b}	1075	0.0000***
L. monocytogenes	15 ± 1.0^{b}	17 ± 0.6^{a}	18 ± 1.0^{a}	10 ± 1.0^{c}	10 ± 1.0^{c}	487.4	0.0000***
Bacillus cereus	20 ± 1.0^{c}	21 ± 0.6^{b}	23 ± 0.6^{a}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	1295	0.0000***
Staph. aureus	35 ± 1.0^{b}	17 ± 1.0^{c}	37 ± 0.6^{a}	13 ± 0.6^{d}	9 ± 1.0^{e}	692.1	0.0000***
Pseudomonas aeruginosa	17 ± 1.0^{c}	19 ± 1.0^{b}	22 ± 0.6^{a}	18 ± 1.0^{bc}	11 ± 0.6^{d}	64	0.0000***
Candida tropicalis	21 ± 1.0^{c}	23 ± 0.6^{b}	27 ± 0.6^{a}	17 ± 1.0^{d}	12 ± 0.6^{e}	169	0.0000***

± Standard error of means

Values with the same letter in the same row are non-significant at p ≤ 0.05

Effect of different nitrogen sources on growth and antimicrobial activity of *S. longisporoflavus*:

Nutrition nitrogen source is known to have a profound effect on the growth rate and production of antimicrobial materials by actinomycetes. The influence of different nitrogen sources as potassium nitrate, ammonium sulphate, sodium nitrate, peptone, calcium nitrate, and tryptone on growth and antimicrobial activity of *S. longisporoflavus* were investigated. The results in table 5 illustrated that among the tested nitrogen sources, maximum mycelial dry weight (0.055 g/ 50 ml) and antimicrobial activity of *S. longisporoflavus* against all the selected MDR bacterial and fungal species were recorded with using potassium nitrate as sole nitrogen source. Also results revealed that the most inhibitory effect of antimicrobial materials produced by *S. longisporoflavus* was recorded with the selected MDR *Staph. aureus* followed by *E. coli, and P. mirabilis* where the diameters of inhibition zones were 37, 35, and 34 mm, respectively.

Table 5. Effect of different nitrogen sources on growth and antimicrobial activity of *S. longisporoflavus*

Nitrogen sources	Nitrogen free	Pot. nitrate	Amm. sulphate	Sod. nitrate	Peptone	Cal. nitrate	Tryptone	F value	P value
S. longisporoflavus dry weight (g/50 ml)	0	0.055	0.023	0.012	0.007	0.04	0.018	709.7	0.0000***
MDR Pathogenic bacterial and fungal Sp.	Inhibition zones diameter (mm)								
E. coli	0.0 ± 0.0^{e}	35 ± 1.0^{a}	15 ± 1.0^{c}	12 ± 1.2^{d}	0.0 ± 0.0^{e}	19 ± 1.0^{b}	15 ± 0.6^{c}	646.8	0.0000***
Proteus mirabilis	0.0 ± 0.0^{f}	34 ± 0.6^{a}	20 ± 1.0^{c}	15 ± 1.0^{d}	13 ± 1.0^{e}	22 ± 0.6^{b}	19 ± 1.0^{c}	417	0.0000***
L. monocytogenes	0.0 ± 0.0^{c}	18 ± 1.0^{a}	10 ± 0.6^{b}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}	10 ± 1.0^{b}	10 ± 0.6^{b}	550	0.0000***
Bacillus cereus	0.0 ± 0.0^{d}	23 ± 1.0^{a}	15 ± 0.6^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	18 ± 1.0^{b}	0.0 ± 0.0^{d}	952	0.0000***
Staph. aureus	0.0 ± 0.0^{e}	37 ± 1.0^{a}	16 ± 0.6^{c}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	28 ± 1.2^{b}	12 ± 1.0^{d}	1280	0.0000***
Pseudomonas aeruginosa	0.0 ± 0.0^{e}	22 ± 1.0^{a}	16 ± 1.0^{b}	9 ± 1.2^{d}	0.0 ± 0.0^{e}	17 ± 1.0^{b}	13 ± 1.0^{c}	282	0.0000***
Candida tropicalis	0.0 ± 0.0^{f}	27 ± 0.6^{a}	21 ± 1.0^{b}	11 ± 1.0^{d}	9 ± 0.6^{e}	22 ± 1.0^{b}	17 ± 1.0^{c}	390	0.0000***

± Standard error of means

Values with the same letter in the same row are non-significant at p ≤ 0.05

Effect of different carbon sources on growth and antimicrobial activity of *S. longisporoflavus*:

In the present study, effect of different carbon sources (glucose, fructose, glycerol, sucrose, starch, maltose, arabinose, and lactose) on the mycelial dry weight and antimicrobial activity of *S. longisporoflavus* are presented in table 6. The results showed that maximum dry weight and diameters of inhibition zones by antimicrobial materials of *S. longisporoflavus* against all the selected MDR bacterial and fungal species, with using starch followed by glycerol and glucose as a carbon source in the culture media. In contrast arabinose, maltose, fructose, sucrose, and lactose exhibited the lowest mycelial dry weight and diameters of inhibition zones with all the selected MDR bacterial and fungal species. The results exhibited that the highest mycelial dry weight of *S. longisporoflavus* and diameters of inhibition zones were remarkedly recorded with the selected MDR *Staph. aureus, E. coli,* and *P. mirabilis* (37, 35, and 34 mm, respectively) with using starch as a carbon source in the culture medium.

Table 6. Effect of different carbon sources on growth and antimicrobial activity of *S. longisporoflavus*

Carbon sources	Carbon free	Glucose	Fructose	Glycerol	Sucrose	Starch	Maltose	Arabinose	Lactose	F value	P value
S. longisporoflavus dry weight (g/50 ml)	0	0.031	0.02	0.043	0.016	0.055	0.029	0.031	0.008	711.4	0.0000***
MDR Pathogenic bacterial and fungal sp.	Inhibition zones diameter (mm)										
E. coli	0.0 ± 0.0^{e}	20 ± 1.0^{c}	11 ± 1.6^{d}	25 ± 1.0^{b}	10 ± 0.5^{d}	35 ± 1.0^{a}	18 ± 1.0^{c}	20 ± 1.0^{c}	10 ± 1.0^{d}	298.1	0.0000***
Proteus mirabilis	0.0 ± 0.0^{e}	15 ± 1.0^{c}	11 ± 1.0^{d}	20 ± 1.0^{b}	10 ± 1.6^{d}	34 ± 0.6^{a}	11 ± 0.6^{d}	14 ± 1.0^{c}	9 ± 1.2^{d}	255.4	0.0000***
L. monocytogenes	0.0 ± 0.0^{c}	13 ± 1.0^{b}	0.0 ± 0.0^{c}	14 ± 1.0^{b}	0.0 ± 0.0^{c}	18 ± 1.0^{a}	0.0 ± 0.0^{c}	13 ± 1.0^{b}	0.0 ± 0.0^{c}	364.8	0.0000***
B. cereus	0.0 ± 0.0^{d}	14 ± 1.0^{c}	0.0 ± 0.0^{d}	18 ± 1.0^{b}	0.0 ± 0.0^{d}	23 ± 1.0^{a}	0.0 ± 0.0^{d}	13 ± 1.0^{c}	0.0 ± 0.0^{d}	594	0.0000***
Staph. aureus	0.0 ± 0.0^{e}	25 ± 0.6^{c}	0.0 ± 0.0^{e}	29 ± 1.0^{b}	0.0 ± 0.0^{e}	37 ± 0.6^{a}	0.0 ± 0.0^{e}	15 ± 1.0^{d}	0.0 ± 0.0^{e}	2325	0.0000***
Pseudomonas aeruginosa	0.0 ± 0.0^{g}	17 ± 1.0^{c}	9 ± 0.6^{f}	19 ± 1.0^{b}	0.0 ± 0.0^{g}	22 ± 1.0^{a}	11 ± 0.6^{e}	13 ± 1.2^{d}	0.0 ± 0.0^{g}	397	0.0000***
Candida tropicalis	0.0 ± 0.0^{f}	23 ± 1.0^{b}	10 ± 0.6^{e}	24 ± 1.0^{b}	10 ± 1.0^{e}	27 ± 1.0^{a}	13 ± 1.0^{d}	19 ± 1.0^{c}	9 ± 0.6^{e}	310	0.0000***

± Standard error of means

Values with the same letter in the same row are non-significant at p ≤ 0.05

DISCUSSION:

Actinomycetes are the most economically and biotechnologically valuable prokaryotes able to produce wide range of bioactive secondary metabolites, such as antibiotics, antitumor agents, immunosuppressive agents, enzymes (Ravikumar *et al*., 2011), cosmetics, vitamins, nutritional materials, herbicides, pesticides, and also well known as a rich source of antibiotics and bioactive molecules (Ogunmwonyi *et al*., 2010). In the present study *S. longisporoflavus* found to produce abroad spectrum antibiotic against several multi-drug resistant pathogenic bacteria and fungi, Rakshanya *et al*. (2011) isolated different actinomycete isolates which were identified as *Streptomyces* sp., *Micromonospora* sp., and *Nocardia* sp., these selected isolates were able to inhibit the growth of *S. aureus, Proteus vulgaris, P. aeruginosa, E. coli, B. subtilis, B. megaterium, K. pneumoniae, C. albicans, A. niger,* and *S. cerevisiae.* Production of secondary metabolites by microorganisms differs qualitatively and quantitatively depending on the strains and species of microorganisms used, as well as of their

nutritional and cultural conditions (Oskay, 2011). Parameters like initial pH, temperature, etc., have a profound effect on the production of bioactive metabolites. In the present study, mycelial dry weight of *S. longisporoflavus* and diameters of inhibition zones of all selected MDR bacterial and fungal species were increased continuously from the 4th to the 8th day, where the maximum dry weight and diameters of inhibition zones were obtained. Further increasing in incubation times showed gradual decrease in the dry weight of *S. longisporoflavus* and the diameters of inhibition zone. This may be because of the fact that antibiotics are secondary metabolites that are produced at the end of the growth phase (Akhurst, 1982), where the decrease in the growth may because of the degradation of the active compounds. Devi *et al*. (2015) reported that the pH and temperature play an important role in growth of *S. erythraea* and erythromycin production. Normally actinomycetes are sensitive to temperature. Initial pH 7.0 was the optimal for mycelial dry weight of *S. longisporoflavus* and its highest antimicrobial activity against all the selected MDR bacteria and fungi, our results are in confirmatory with Wang *et al*. (2010) who reported that the optimal primary pH was around 7.0 for production of compounds with antimicrobial activity by actinomycete strain Hhs.015T. Sujatha *et al*. (2005) found that maximum antibiotic production activity was obtained at a pH of 7.2, suggesting its inclusion in the neutrophilic actinomycetes group. The present results also revealed that dry weight of *S. longisporoflavus* and diameters of inhibition zones of all selected MDR bacterial and fungal species increased as the incubation temperature increase up to 55°C at which the highest activity was recorded ,and then the activity decreased, similar results were obtained by Yabe *et al*. (2011) who found that, a thermophilic, strain belong to the family Nocardioidaceae, designated I3T, grew at temperatures between 35 and 62ºC, with optimum growth at 50 – 55ºC. The nutritional sources of carbon and nitrogen are known to have a profound effect on the antimicrobial compound production by actinomycetes (Himabindu and Jetty, 2006). It was observed in the present study that, the highest mycelial dry weight of *S. longisporoflavus* and diameters of inhibition zones of all selected MDR bacterial and fungal species were recorded by using starch as a carbon sources followed by glycerol and glucose and with using potassium nitrate as a nitrogen source followed by calcium nitrate. These results are in agreement with Al-Zahrani (2007) who reported that maximum antibacterial agents biosynthesis was obtained in medium supplemented with starch as a sole carbon source followed by glucose and with potassium nitrate as nitrogen source.

Conclusion:

Antimicrobial materials from actinomycetes are alternative drug resistant to chemical drugs in controlling the human pathogenic bacteria and fungi. In the present study *S. longisporoflavus* was screened for its antimicrobial activity against fourteen MDR pathogenic bacterial and fungal species. The results exhibited abroad spectrum of activity against six MDR pathogenic bacteria and one fungal species. After optimizing culture conditions of *S. longisporoflavus* the results showed significant improvement in antimicrobial activity of *S. longisporoflavus* against the tested MDR *Staph. aureus, E. coli, and Proteus mirabilis* which are the most important pathogenic bacterial species to human.

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ضبط ظروف النمو والنشاط الضد ميكروبي لأستربتوميسس لونجيسبوروفلافس

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تم في هذه الدراسة اختبار النشاط الضد ميكروبي للمواد الضدة الميكروبية الناتجة من استربتوميسس لونجيسبوروفلافس علي بعض انواع البكتريا (استافيلوكوكس اوريس، ايشريشيا كولاي، بروتيس ميرابيليس، بسيدوموناس ايروجينوزا، باسلس سيرس، ليستيريا مونوسيتوجينس، كليبسيلا نومونيا، سلمونيلا تيفيميوريم) والفطريات(تريكوسبورن ميوكويدز، كانديدا جليبراتا، كانديدا كروزي، كانديدا البيكانز، كانديدا تروبيكاليز، كانديدا دابلينيسيز) الممرضة المقاومة للمضادات الحيوية وذلك بإستخدام طريقة الانتشار خلال الاجارز وقد اظهرت المواد الضد ميكروبية لاستربتوميسس لونجيسبوروفلافس نشاط ضد ميكروبي ضد ايشريشيا كولاي، بروتيس ميرابيليس، بسيدوموناس ايروجينوزا، استافيلوكوكس أوريس، باسلس سيرس، ليستيريا مونوسيتوجينس، كانديدا تروبيكاليسز وقد تم ضبط ظروف زراعة استربتوميسس لونجيسبوروفلافس لتزويد النمو والنشاط الضد ميكروبي. وقد سجلت النتائج تحت الظروف المثلي اقصي وزن جاف واقصي نشاط ضد ميكروبي لاستربتوميسس لونجيسبوروفلافس علي كل الانواع البكتيرية والفطرية المختارة المقاومة للمضادات الحيوية وذلك عند فترة تحضين 8 أيام ورقم هيدروجيني 7 وحرارة 55 م° ووسط غذائي مزود نترات بوتاسيوم كمصدر نيتروجيني ونشا كمصدر كربوني. وقد سجلت النتائج ان اعلي مقياس لأقطار مناطق التثبيط بواسطة المواد الضد ميكروبية الممرضة المقاومة للمضادات الحيوية استافيلوكوكس أوريس متبوعه ايشريشيا كولاي وبروتيس ميرابيليس (37، 35، 34مم بالتتابع) تحت كل الظروف المثلي لنمو استربتوميسس لونجيسبوروفلافس.