Endophytic *Streptomyces olivaceiscleroticus* Endo-1: Biocontrol Agent and Growth Promoter of Wheat

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Seven endophytic actinobacterial isolates were isolated from wheat roots, which collected from the middle area of Riyadh at Saudi Arabia. The obtained results revealed that, all endophytic isolates have the ability to produce the growth hormone indole acetic acid (18.2 - 30.7 μg.ml^{-1}). *In vitro* antifungal activity of the seven endophytes was investigated against the phytopathogenic fungus *Fusarium graminearum*. Out of these, the isolate Endo-1 showed the maximum fungal inhibition. The optimum cultural conditions were studied to obtain the highest *in vitro* antifungal activity of Endo-1 isolate. It was found that the first incubation day, pH 5.5, incubation temperature 30°C, starch as the best original carbon source, and peptone as the best substitutive nitrogen source were the best conditions for maximum inhibitory effect. On the other hand, Endo-1 isolate was tested under greenhouse conditions to determine its efficacy in the biocontrol of *Fusarium* head blight disease of wheat. The data showed that treatment with Endo-1 isolate significantly reduced both disease severity and incidence and improved all assessed growth parameters when compared with the control treatment. Endo-1 isolate was identified as *Streptomyces olivaceiscleroticus* using the morphological, physiological and biochemical characteristics and the identification was confirmed molecularly using 16S rRNA. Thus this endophytic isolate has the potential as plant growth promoters as well as a bio-control agent, which is a useful trait for crop production in nutrient deficient soils.

Key words: Endophytic *Streptomyces*, Biocontrol, *Fusarium* Head Blight disease and Wheat.

Triticum aestivum L is the most common and important cereal crop which has high yield and quality for food and feed production, so it is called "bread wheat". *Fusarium* Head Blight (FHB), caused by *Fusarium graminearum*, is an important disease of wheat throughout the world's wheat-growing areas. This disease primary infects the anthers where spores of fungus land and then grow into the kernels, glumes, or other head parts. Some evidences suggest that, wheat may be susceptible in a period up through the soft dough stage of kernel development[1]. The grain from head-blighted fields is unsuitable for feeding farm animals as it may contain mycotoxins that induce vomiting in animals. Bread made from scabby wheat has been reported to be intoxicating[2].

Endophytes are internal colonized microbes which can live symbiotically with the healthy plants. Strict surface-sterilized methods can use for isolation of these microbes from plant tissues[3]. Endophytes have a closer relationship with plant tissues more than rhizospheres. Streptomycetes are a major group of plant endophytic microbes and have been found to produce various active metabolites. *Streptomyces* is a most common and wide distributed genus of this group which has been isolated from various

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surface-sterilized plant tissues and some species have shown *in vitro* intensive antifungal activities[4]. A novel antifungal antibiotic was produced by Endophytic *Streptomyces* from rhododendron plant, fistupyrone which protected Chinese cabbage from infection by *Alternaria brassicicola*[5]. Tokata *et al.,*[6] stated that, *Streptomyces lydicus* WYEC108, could be colonized inside the nodules of pea plants, and increased root nodulation and improved the vigour of bacteroids in the nodules, due to the enhanced nodular assimilation of iron by the *Streptomyces*. In contrast, some endophytic *Streptomyces* synthesize cytokinin-like metabolites that inhibit rice seed germination and rooting[7]. Studies on these bioactive metabolites will be useful in exploiting the relationships between endophytic *Streptomyces* and their host plants, or discovering novel agrochemical precursors.

Endophytic actinobacteria have been isolated from *Triticum aestivum* L and have been shown to have a number of beneficial effects on the plant when reintroduced and they are reported to control fungal infections in wheat[8, 9]. A highest number of the active isolated endophytes belong to the actinobacterial phylum and, specifically, the genus *Streptomyces*[8, 10].

The present study is aimed to investigate the *in vitro* antifungal activity against *F. graminearum* and the biocontrol and plant growth-promotion activities of endophytic actinobacteria in wheat plants against FHB disease.

MATERIALS AND METHODS

Wheat samples collection

The roots of five healthy wheat plants were collected from wheat plantations located in the Middle area, Riyadh, Saudi Arabia. Samples were placed in plastic bags, which were taken to the laboratory and processed within 4 h after collection.

Isolation of the pathogen

The fungal pathogen *F. graminearum* was isolated from naturally diseased wheat plant exhibiting typical symptoms of FHB disease. Purifica-tion of the isolated fungi was done using single spore technique. The pathogen was identified as *F. graminearum* based on the cultural properties, morphological and microscopi-cal characteristics as described by Booth[11].

Surface sterilization and actinomycetes isolation

Wheat roots (0.5–1.0 cm in diameter) were washed under running tap water to remove soil particles, and then sterilized by sequential immersion in 70% ethanol for 5 min and a solution of sodium hypochlorite (0.9% available chlorine) for 20 min. Samples were washed by sterile water three times to remove the residues of sterilizing agents; and then soaked in 10% $NaHCO_3$ solution to disrupt the plant tissues and inhibit the growth of fungi. Each sample was divided into small fragments and placed on starch nitrate agar medium[12] with 15 µg/ml^{-1} nalidixic acid to suppress the growth of bacteria, and incubated at 30°C[3].

Extraction and identification of indole acetic acid (IAA)

Seven endophytic actinobacterial isolates were grown on non-fat milk broth (NFM) medium containing tryptophan (1.0 mg.L^{-1}) and NH_4Cl (1.0 g.L^{-1}). The cultures were grown for 72 h at 30°C in a water bath shaker. The bacterial cells were separated by centrifugation at 10,000 rpm for 15 min. The pH of supernatant was adjusted at 2.8 with HCl and then extracted 3 times with equal volume of ethyl acetate[13]. The extract was evaporated to dryness and residue was resuspended in 2 ml of ethanol. The samples were analyzed on HPLC using UV-detector and Tech sphere 5-ODS C-18 column. The methanol: acetic acid: water (30:1:70) mixture was used as mobile phase with flow rate 1.5 ml/min. For identification, 20µl sample, filtered through a 0.45µm filter, was injected into the column. Identification of IAA was done on the basis of retention time of the standard IAA by using a refractive index detector (RI). The concentration was calculated on the basis of peak height and peak area in comparison with standard.

In vitro antifungal activity

In vitro growth inhibition of *F. graminearum* by the endophytic actinobacteria was tested on potato dextrose agar media. For each actinobacterial isolate, 1 ml of the suspension (10^8 CFU/ml) was poured on the margin of agar plates and 6 mm agar disc of fungus from fresh PDA culture was placed at the other marginal side and incubated at 25 ± 2°C for seven days. The radii of the fungal colony towards and away from the actinobacterial colony were noted. The growth inhibition percentage was calculated using the

following equation:

$\% \text{ Inhibition} = [(R - r) / R \times 100]$

Where, r is the radius of the fungal colony opposite the bacterial colony and R is the maximum radius of the fungal colony away from the bacterial colony.

Culture conditions optimization

Optimum culture conditions were studied to obtain the highest *in vitro* antifungal activity of actinobacterial isolate Endo-1 against *F. graminearum*.

Effect of incubation period and pH value

There were seven Erlenmeyer flasks (250 ml) used; each of which contained 100 ml of starch nitrate broth. Medium pH for each flask was adjusted at definite value (5, 5.5, 6, 6.5, 7, 7.5 and 8). The flasks were autoclaved, inoculated by equal inoculum size of Endo-1 isolate, and then incubated in the incubating shaker (160 rpm) at 30 ± 1°C for various incubation periods (1, 2, 3, 4, 5, 6 and 7 days). Antifungal activity was assayed by using the inhibition zone method at each incubation period and pH value[14].

Effect of incubation temperature

There were seven Erlenmeyer flasks (250 ml) contained 100 ml of starch nitrate broth. Medium pH for each flask was adjusted at the optimum value 5.5, inoculated, and then incubated for 24 h as the best incubation period in the incubating shaker (160 rpm) at different temperatures (24, 26, 28, 30, 32, 34 and 36°C). Antifungal activity was assayed by using the inhibition zone method at each incubation temperature[14].

Effect of carbon and nitrogen sources

The effect of different carbon sources on the antifungal activity of Endo-1 isolate was studied by using starch nitrate broth medium. Starch nitrate broth contained starch as the main carbon source, which was substituted with different sugars as alternative carbon sources. These sugars are D-glucose, D-fructose, and D-galactose as monosaccharides (0.132 g/100 ml); sucrose, lactose, and maltose as disaccharides (0.07 g/100 ml) and ribose (0.158 g/100 ml). Eight Erlenmeyer flasks were prepared, each one contained 100 ml of the medium (KNO_3 0.2g; $CaCO_3$ 0.3g; KH_2PO_4 0.1g; NaCl 0.05g; $MgSO_4.7H_2O$ 0.05g), each medium was supplemented with certain carbon source as mentioned previously. The pH of all media was adjusted at 5.5. All flasks were autoclaved, inoculated with Endo-1 isolate, and incubated for 24 h at 30°C in the incubating shaker (160 rpm). Antifungal activity was assayed using the inhibition zone method at each carbon source. To determine the best nitrogen source, the same medium was used but potassium nitrate (original nitrogen source of starch nitrate medium) was substituted with different chemical and natural nitrogenous products as alternative nitrogen sources. These nitrogenous products are casein (0.006 g/100 ml), peptone, yeast extract, malt extract and beef extract as natural nitrogenous products, which have the same weight of potassium nitrate in the composition of starch nitrate medium (0.2 g/100 ml); ammonia (0.05 g/100 ml) and ammonium sulfate (0.2 g/100 ml) as chemical nitrogenous products. The same previous procedure was applied, however, medium composition is (Starch 2.0g; $CaCO_3$ 0.3g; KH_2PO_4 0.1g; NaCl 0.05g; $MgSO_4.7H_2O$ 0.05g). Antifungal activity was assayed using the inhibition zone method at each nitrogen source[15].

Identification of actinomycete isolate

The morphological and cultural characteristics of the actinomycete isolate Endo-1 were studied according to Shirling and Gottlieb[16]. For electron microscopy, ISP4 agar medium was inoculated with spores of actinomycete isolate and incubated for 7 days at 28°C. A plug of the culture was removed and fixed in glutaraldehyde (2.5% v/v), washed with water and post-fixed in osmium tetraoxide (1% w/v) for 1 h. The sample was washed twice with water and dehydrated in ascending ethanol before drying in a critical point drying apparatus (Polaron E3000) and finally coated in gold and examined in a JEOLISM 541OLV scanning electron microscope at 15 kv. Identification of the selected isolate was confirmed using 16S rRNA sequencing. 16S rRNA was amplified in a thermocycler (Perkin Elmer Cetus Model 480) by using universal primers of 27f (5'-AGA GTT TGA TCC TGG CTC AG -3') and 1525r (5'-AAG GAG GTG ATC CAG CC-3') under the following condition: 94°C for 5 min, 35 cycles of 94°C for 60s, 55 for 60s, 72°C for 90s and final extension at 72°C for 5 min. The product was directly sequenced by a BigDye terminator cycle sequencing kit (PE Applied Biosystems USA) on an ABI 310 automated DNA sequencer (Applied Biosystems, USA). Homology of the 16S rRNA sequence of

isolate was analyzed by using BLAST program from GenBank database[17].

Preparation of actinobacterial suspension

Two Erlenmeyer flasks (1000 ml) containing 400 ml of starch-nitrate broth were autoclaved and then inoculated with a 14-day-old colony of Endo-1 isolate grown on starch nitrate slants. The medium was adjusted at pH 5.5. The inoculated flasks were then incubated in the incubating shaker at 30°C and 160 rpm for 24 h.

In Plant a biocontrol activity of endophytic actinobacteria

Surface-sterilized wheat seeds were soaked in the Endo-1 suspension for 1 h with occasional shaking to ensure uniform coating on the surface under aseptic conditions. Soaked seeds in sterilized distilled water were treated as control. The seeds were allowed to grow in Petri plates having autoclaved filter paper, at 20°C for 6 days in growth cabinet. One–week-old seedlings were then transplanted in plastic pots containing sterilized soil. Plants were watered with 1/4th Hoagland solution when required. Four plants were maintained in each pot and placed in a growth chamber under standard conditions (18 h light, 25 ± 2°C and 60% relative humidity). After one week of transplantation, 15 ml of spore suspension (10^5 spore.ml^{-1}) of *F. graminearum* were sprayed on the wheat plants to obtain the infection. Some pots were treated with 15 ml of spore suspension of Endo-1 isolate as a soil drench. Plants containing neither pathogen nor endophytic actinobacterial isolate were treated as negative control. Plants were harvested after six weeks for assessment of disease severity and growth parameters (root and shoot length, fresh and dry weight). Triplicates were used for each treatment. All pots were arranged in a complete randomized design.

Disease severity assessment

After harvesting, disease severity and incidence were assessed. Disease severity was rated by visual scaling ranging from 0 to 5. A rating of 0 means no evidence of infection, and rating of 1, 2, 3, 4 and 5 reflected an infected surface area of appropriately 5, 25, 50, 75, and 99 -100% respectively.

$$\text{Disease severity} = \frac{\Sigma(ab) \times 100}{AK}$$

Where:

a = No. of diseased plants having the same degree of infection

b = Degree of infection

A = Total no. of examined plants

K = Highest degree of infection

RESULTS

Isolation and characterization of the endophytic actinobacterial isolates

Seven endophytic actinobacterial isolates were isolated from the surface sterilized wheat roots on starch nitrate agar medium. Microscopic examination of the obtained isolates showed that all isolates are Gram positive bacteria. Three of them (Endo-1, Endo-2, and Endo-3) have spiral spore chains and gray aerial mycelia. Colonies of the isolates (Endo-1 and Endo-2) have regular shape with crenate boarders, while that of Endo-3 isolate has irregular shape of colonies with swarming growth. The two isolates Endo-4 and Endo-5 have rod shaped spore chains. Colony of Endo-4 is white irregular with swarming growth, while that of Endo-5 is green irregular with wrinkled surface. The isolates Endo-6 and Endo-7 have hook shaped spore chains and irregular colonies with wrinkled surfaces. The colony color of Endo-6 is

Table 1. Morphological and cultural characteristic of endophytic actinobacterial strains

Isolate	Gram stain	Spore chain	Colony color	Colony shape
Endo-1	Positive	Spiral	Gray	Regular/crenate boarders
Endo-2	Positive	Spiral	Gray	Regular/circular boarders
Endo-3	Positive	Spiral	Gray	Irregular/ swarming growth
Endo-4	Positive	Rod	White	Irregular/ swarming growth
Endo-5	Positive	Rod	Green	Irregular/ wrinkled surface
Endo-6	Positive	Hook	Red	Irregular/ wrinkled surface
Endo-7	Positive	Hook	Yellow	Irregular/ wrinkled surface

Table 2. Effects of incubation periods and pH values on the antifungal activity of Endo-1isolate

Incubation period (day)	Inhibition zone (mm) pH value						
	5	5.5	6	6.5	7	7.5	8
1st	1	4.2	3	3.2	2.2	3.7	2.5
2nd	1	3	1.8	1	2.5	2.2	0
3rd	1	2.5	2.5	0.8	1	1.8	0
4th	0	2.2	0.	0	1	0	0
5th	1	1.5	1.5	2.2	2.2	2.4	3
6th	1	1	1	1.2	1	1.5	2.1
7th	0	0	0	0	0	0	0

red while Endo-7 is yellow (Table 1).

Indole acetic acid production

Indole acetic acid production test revealed that, all isolates have the ability to produce IAA. Endo-1 isolate recorded the highest yield of IAA followed by Endo-2 isolate (30.7, 25.4 $\mu g.ml^{-1}$, respectively), whilst, the lowest production was achieved by the isolate Endo-7 (18.2 $\mu g.ml^{-1}$). Production of IAA by the other isolates was ranged between 23.1 - 18.27$\mu g.ml^{-1}$ (Fig. 1).

***In vitro* antifungal activity assessment**

Antifungal activity of the seven endophytic actinobacterial isolates was investigated *in vitro* against *F. graminearum* to determine the most potent antifungal isolate. Out of the seven isolates, Endo-1 showed the highest inhibition percentage 80% against *F. graminearum*, while Endo-7 was the lowest recording 35% inhibition percentage (Fig. 2). Based on the obtained results, Endo-1 isolate was selected for next tests.

Optimization for maximum antifungal activity of Endo-1 isolate

Effect of incubation period and pH value

The optimum cultural incubation period and initial pH value effects were studied synchronously to obtain the highest *in vitro* antifungal activity of actinobacterial isolate Endo-1 against *F. graminearum* (Table 2). The results revealed that, the first day of incubation at pH 5.5 recorded the highest antifungal activity as the best incubation period and initial pH value. According to the obtained, the antifungal activity of isolate Endo-1 has a wide range of pH (5-8) and incubation time (1-6 days). In contrast, no antifungal activity was observed at 7th day of incubation at any pH value.

Effect of incubation temperature

The obtained results showed that, the highest antifungal activity was recorded at 30°C. The antifungal activity was first noticed at 24°C,

Table 3. Disease severity and incidence in wheat plants

Treatment	Disease severity	Disease incidence (%)
Control	0	0
Endo-1	0	0
Pathogen	70.4	86.4
Endo-1+ Pathogen	48.2	54.6

Table 4. Growth Parameters of wheat plants

Treatment	Length (cm)		weight (gm)			
			Shoot		Root	
	Shoot	Root	Fresh	Dry	Fresh	Dry
Control	33	19	5.0	2.8	2.2	0.4
Endo-1	40	22	7.2	4.2	2.8	0.6
Pathogen	27	12	3.7	1.8	1.7	0.2
Endo-1+ Pathogen	30	17	4.2	2.2	2.0	0.3

then increased gradually until 30°C as the best temperature, and then started to decline gradually and disappeared completely at 36°C (Fig. 3).

Effect of carbon and nitrogen sources

Our results revealed that, starch was the best carbon source, at which the highest antifungal activity was observed followed by sucrose, D-fructose and D-galactose, D-glucose, ribose, lactose and maltose (Fig. 4). On the other hand, peptone was the best nitrogen source, at which the highest antifungal activity was achieved followed by yeast extract, beef extract, ammonium sulfate, potassium nitrate, casein, ammonia and malt extract (Fig. 5).

Disease assessment

The pots experiment was conducted under the greenhouse conditions. The results of disease severity and incidence were presented in Table 3. The data showed that treatment with Endo-1 isolate significantly reduced both disease severity and incidence when compared with the control treatment (48.2 and 54.6%, respectively). No disease symptoms were observed in both of control and End-1 treatments.

Table 5. Morphological, physiological and biochemical characteristics of Endo-1isolate

Characteristic	Item	Result
Morphological	Spore mass	Gray
	Spore surface	Smooth
	Spore chain	Spiral
	Color of substrate mycelium	Grayish yellow
	Diffusible pigments	Not produced
Biochemical	Diaminopimelic acid	LL-DAP
	Sugar pattern	Not detected
Enzymatic activity	Hydrolysis of protein, lipid, starch and egg yolk (lecithin)	+
	Catalase, urease and protein coagulase test	-
Melanoid pigment	Peptone yeast extract iron agar medium	-
	Tyrosine agar medium	-
	Tryptone-yeast extract broth medium	-
Matters degradation	Esculin	+
	Xanthin	+
Physiological	H_2S production	-
	Nitrate reduction	+
	Citrate utilization	+
Utilization of carbon sources	D-xylose, D-mannose, D-galactose and trehalose	+
	D-glucose, mannitol, lactose and maltose	++
	L-arabinose, raffinose and rhamnose	-
	Starch, D-fructose and Sucrose	+++
Utilization of nitrogen sources	L-cystiene, L-histidin, L-phenylalanine, L-lysine	+
	L-valine	-
	L-arginine, L-serine, L-tyrosine	++
Growth with	Thallus acetate (0.001)	+
	Sodium azide (0.01)	+
	Phenol (0.1)	+
Environmental factors	Growth temperature	30°C (24-40 °C)
	Optimum pH	5.5 (5.0-8.5)
Antibiotics resistance	Ampicillin (10 ug) and Erythromycin (15 ug)	+
Antimicrobial activity	*Staphylococcus. aureus* NCTC 7447	-
	Pseudomonas aeruginosa ATCC 10145	-
	Saccharomyces cerevisiae ATCC 9763	+
	Aspergillus. niger IMI 31276	+

+ = Positive, - = Negative, ++ = moderate growth, +++ = good growth results

Effects on growth parameters

Treatment with Endo-1 isolate improved all assessed growth parameters (root and shoot length, fresh and dry weight) compared to the control treatment. On the other hand Infection with the fungal pathogen reduced all growth parameters. Data in Table 4 showed that the reduction effect in the all growth parameters due to infection was lower in the infected wheat plants when treated with Endo-1 suspension.

Identification of actinobacterial isolate Endo-1

Identification results revealed that, the aerial mycelia can grow abundantly on starch nitrate agar; oatmeal agar (ISP-3) and glycerol-asparagine agar media (ISP-5). Scanning electron microscopy showed that spore chains are in spiral shape with smooth surface (Fig. 6). Sclerotic granules were formed on some media, whereas no sporangia were being observed. The biochemical analysis of cell wall revealed the presence of LL-diaminopimelic acid (LL-DAP) while sugar pattern was not detected (Table 5). It was found that, Endo-1 isolate can produce protease, lipase, amylase and lecithinase enzymes, while catalase, urease and protein coagulase enzymes are not. The physiological results showed that, no melanoid pigment was produced by Endo-1 isolate after growing on three specific media; peptone yeast extract iron agar, tyrosine agar and tryptone yeast extract broth. The results of biodegradation process showed that, esculin and xanthin were degraded, hydrogen sulfide was not formed, nitrate was completely reduced to nitrite, and citrate was utilized. Ultimately, Actinobacterial isolate Endo-1 could not utilized L-arabinose, raffinose and rhamnose, while it had good growth with starch, sucrose and D-fructose, moderate growth with D-glucose, mannitol, lactose and maltose, and weak growth with D-xylose, D-mannose, D-galactose and trehalose. Endo-1 isolate utilized amino acid L-valine while it had moderate growth on L-arginine, L-serine and L-tyrosine, and weak growth on L-cystiene, L-histidin, L-phenylalanine and L-lysine. This isolate was found to have weak growth on some toxic substances such as thallus acetate (0.001), sodium azide (0.01) and phenol (0.1). Actinobacterial isolate Endo-1 was found to have resistance to beta lactam antibiotics represented by ampicillin (10 µg) and aminoglycosides represented by erythromycin (15 µg). Endo-1 isolate was found to have antifungal activity against *Saccharomyces cerevisiae* ATCC 9763 and *Aspergillus niger* IMI 31276, while it did not has antibacterial effect against neither Gram-positive nor Gram-negative; such as *Staphylococcus aureus* NCTC-7447 and *Pseudomonas aeruginosa* ATCC-10145 respectively.

Color and culture characteristics

It was found that, the isolate Endo-1 has gray aerial mycelia; grayish yellow substrate mycelia and the diffusible pigment is not produced (Table 6). This was performed basically according to the recommended international Key's viz. the color of the organism under investigation was consulted with the ISCC-NBS color-name charts illustrated with centroid color. On the basis of the previously collected data and in view of the comparative study of the recorded properties of

Table 6. Culture characteristics of the actinomycete isolate endo-1

Medium	Growth	Mycelia		Diffusible
		Aerial	Substrate	pigment
Starch nitrate agar medium	Good	264-L.Gray	gy-y 90	76-l-y-br
Tryptone yeast extract broth (ISP-1)	No	No	No	No
Yeast-malt extract agar medium (ISP-2)	No	No	No	No
Oat-meal agar medium (ISP-3)	Moderate	L.Gray264	gy-y 90	-
Inorganic salts starch agar medium (ISP-4)	Good	L.Gray264	gy-y 90	-
Glycerol-Asparagine agar medium (ISP-5)	Good	L.Gray264	gy-y 90	-
Peptone yeast extract iron agar medium (ISP-6)	Moderate	Gray264	57-1.br	58 m-br
Tyrosine agar medium (ISP-7)	Poor	L.Gray264	57-1.br	58 m-br
Potato dextrose agar medium	Good	L.Gray264	gy-y 90	-
Glucose casein agar medium	Moderate	L.Gray264	gy-y 90	-

L.Gray = light gray, gy-y = grayish yellow, l-y-br = light yellowish brown, 1.br = light brown, m-br = moderate brown

Endo-1 in relation to the most closest reference strain, viz. *Streptomyces olivaceiscleroticus*, it could be stated that actinomycetes isolate, Endo-1 is suggestive of being likely belonging to *Streptomyces olivaceiscleroticus*. When the 16S rRNA gene from the isolate Endo-1 was sequenced, it showed a 98% similarity with sequences from *S. olivaceiscleroticus* strains. The strain was designated as *S. olivaceiscleroticus* Endo-1.

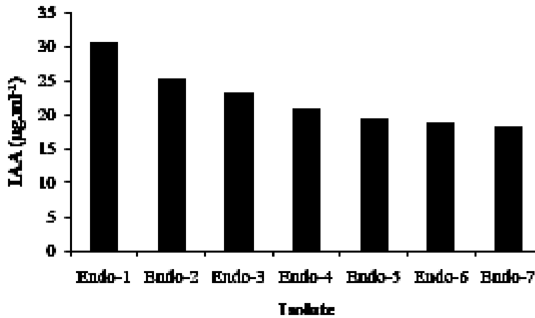

Fig. 1. Production of IAA (µg.ml^{-1}) of seven actinobacterial isolates

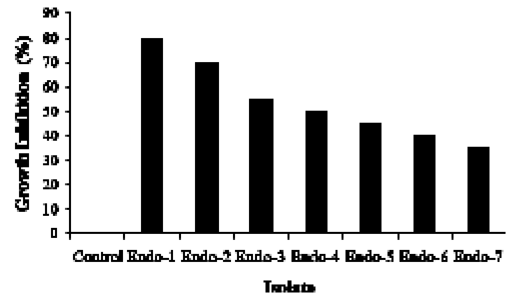

Fig. 2. *In vitro* growth inhibitions of *F. graminearum* by actinobacterial isolates

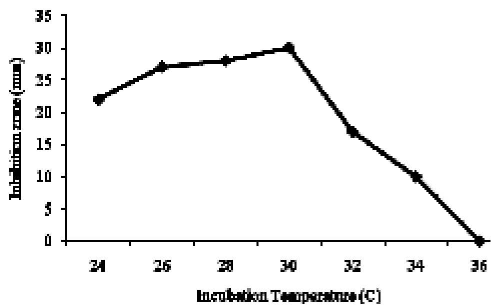

Fig. 3. Effect of different incubation temperatures on the antifungal activity of End0-1 isolate

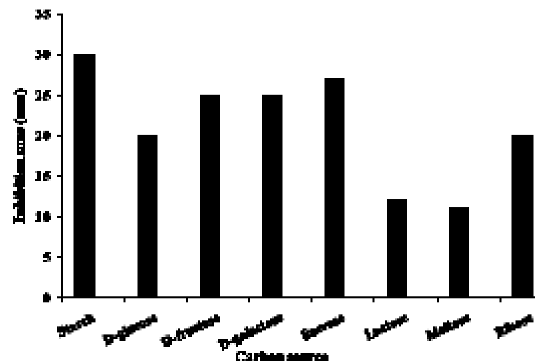

Fig. 4. Effect of different carbon sources on the antifungal activity of Endo-1 isolate

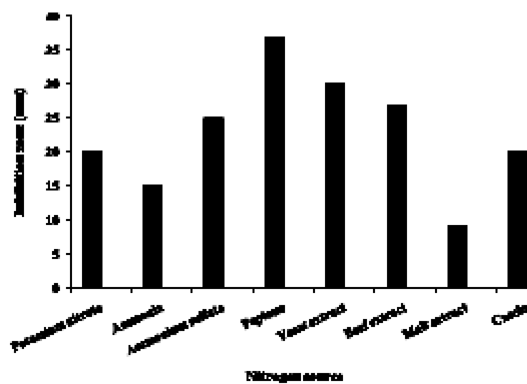

Fig. 5. Effect of different nitrogen sources on the antifungal activity of Endo-1 isolate

Fig. 6. Scanning electron micrograph of *Streptomyces olivaceiscleroticus* Endo-1

DISCUSSION

Seven endophytic actinobacteria were isolated from surface-sterilized wheat roots. These isolates showed *in vitro* antifungal activity against *F. graminearum* and respectable ability to produce the plant growth hormone IAA. Several recent studies demonstrated that, endophytic actinomycetes produced plant growth-promoting compounds such as IAA (18, 19, 20, 21). Other studies proved that, endophytic actinobacteria could be expressed antifungal activity against wide scale of soil-borne fungi by producing high levels of cell-wall degrading enzymes, such as β-1,3; β-1,4 and β-1,6-glucanases[4, 22]. Some endophytic microbial flora included actinobacteria can provide their host with protection against phytopathogenic fungi, and consider one of defense lines inside the plant. On the other hand, these endophytes aid in the plant growth promotion by production of IAA, which in turn absorbed by plant tissues and accumulates in plant cells to promote the growth. So, these endophytic actinobacteria provide dual benefit for their host (wheat).

The results of greenhouse experiment revealed that, *S. olivaceiscleroticus* Endo-1 isolate has significant biocontrol effect against FHB disease. Moreover, treatment with *S. olivaceiscleroticus* Endo-1 isolate led to increase the tested growth parameters of wheat. Nourozian *et al.,*[23] reported that, *Streptomyces* strain 3 was found has significant biocontrol effect against *F. graminearum* and increased the yield of wheat compared with the un-inoculated control. *In vitro* inhibitory activities of the antagonistic strains do not always allow an accurate prediction of disease control potential. Schottel *et al.,*[24] used spontaneous mutants of antagonistic streptomycete strains to assess the importance of antagonism in the biocontrol of potato scab. Despite a reduced *in vitro* pathogen inhibition activity, most of these mutants demonstrated significant biocontrol activity against potato scab. On the other hand, bacterial influence on plant growth may be an important determinant in biocontrol. In our preliminary experiments we have observed similar dependence between plant growth promotion and increased disease resistance. In this case, direct enhancement of wheat growth by *Streptomyces* may be one of the key mechanisms by which *Streptomyces* antagonists enhance plant health. Upon infection by certain endophytic streptomycetes, plants acquire an increased resistance to pathogen attack. This phenomenon has been classified as priming[25]. Rhizosphere and endophytic streptomycetes have been recently indicated as such disease resistance inducing species. Cell wall thickenings, production of some phenolic compounds, production of siderophore, β-1,3 glucanase, chitinase, antibiotics, cyanide and expression of some defense genes may be some of the mechanisms by which the colonized plant can face the pathogen[26].

Optimum cultural conditions were studied to obtain maximum antifungal activity of *S. olivaceiscleroticus* Endo-1 against *F. graminearum*. The results showed that, the highest *in vitro* antifungal activity was observed at the first day as the best incubation period and initial pH 5.5. These results are in agreement with that of Mansour and Mohamedin[27] whom found the highest fungi cell-wall lytic activity of *S. thermodiastaticus* was obtained after 18 h of incubation and at pH 5.5. Most of the antifungal compound(s) are sensitive and degrade by acidity; although, this strain produces acid-fast antifungal compound(s), which inhibit the pathogen in the acidic environment, which form due to accumulation of extracellular metabolites during the growth outside the microorganism. The best incubation temperature was observed at 30°C. This result indicates that, *S. olivaceiscleroticus* Endo-1 is a mesophilic actinobacterium, which grows and produces antifungal activity at moderate temperatures, typically like inside the plant. de Oliveira *et al.,* (19) reported that, *S. pluricolorescens* was found has highest inhibitory effect against *F. oxysporum* var. *lycopersici* at 30°C.

In the present study, starch was the best carbon source at which highest antifungal activity was observed. This result may seems contrary to logic, because starch is complex polysaccharide molecule, which needs more time and energy to degrade and converts to easy absorbed monosaccharides; but starch molecule has a multitude numbers of glucose molecules as building blocks which be free in the medium after degradation of starch more than which supplemented directly to the medium. These

monosaccharides in turn use in the energy formation, which utilize in growth and production of antifungal compound(s) processes. Tawfik and Ramadan[28] reported that starch is considered to be the best carbon source for growth and excretion of antibiotic by two *Streptomyces* strains. On the other hand, peptone was the best nitrogen source at which highest antifungal activity was observed against *F. graminearum*. Peptone is much enriched natural product by nitrogen, which included in the short chains of amino acids monomers, which are easy during degradation and absorption by the microorganism more than other chemical and natural nitrogenous compounds. Neha and Vibhuti[29] found that, peptone was the best nitrogen source for the highest antifungal activity and biomass production of oxytetracycline by a strain of *S. rimosus* 9306027.

Therefore, it can be concluded that *S. olivaceiscleroticus* Endo-1 has the potential to be a biocontrol agent for FHB in wheat plant. However, further fieldwork is required to confirm their control efficacy in different climatic regions and under different growth conditions. Formulation and applications that meet common farming practices still need to be developed. For promising biological control agents, strategies that enhance overall control efficacy should be explored.

CONCLUSION

There was an endophytic actinobacterium (Endo-1) isolated from surface-sterilized wheat roots. This isolate was found has antifungal activity against *Fusarium graminearum* as the main cause of FHB disease for wheat. Optimum cultural conditions were studied to obtain a maximum antifungal activity. On the other hand, Endo-1 isolate was showed powerful growth promotion effect for wheat due to its high ability for production of plant growth hormone IAA (30.7 $\mu g.ml^{-1}$). Endo-1 isolate was identified as *Streptomyces olivaceiscleroticus*; by using classical and molecular methods as mentioned above.

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