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ENDOPHYTIC BACTERIA ASSOCIATED WITH SUGARCANE AND ITS POTENTIAL TO SUPPRESS *SPORISORIUM SCITAMINEUM*, THE CAUSAL FUNGAL PATHOGEN OF SMUT DISEASE

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ABSTRACT

Bacterial endophytic ubiquitously colonize the internal tissues of the plant. The purpose of this study was to isolate and identify the endophytic bacteria from sugarcane stalks which have antagonistic activity against *Sporisorium scitamineum,* the causal pathogen of sugarcane smut disease, and to study their activity to produce some secondary metabolites i.e. enzymes and growth-promoters. Sugarcane samples were collected from five governorates in Upper-Egypt namely, Giza, Beni-Suef, Sohag, Qena, and Luxor. A total of 240 isolates of endophytic bacteria were isolated from 160 samples of healthy sugarcane stalks (variety GT-54-9). Isolated endophytes were screened for its antagonistic activity against *S. scitamineum, in vitro*, using dual culture method. Only, 62 isolates showed different degrees of antagonistic activity. Ten isolates of endophytic bacteria were selected to study their potential to produce enzymes (chitinase, β 1,3 glucanase, phenylalanine ammonia lyase) and growth-promoters (siderophores, indole acetic acid (IAA), salicylic acid (SA)). These isolates were pre-identified using the morphological and physiological properties according to Bergy's manual as *Pseudomonas* sp., *Serratia* sp., *Enterobacteria* sp., *Herbaspirillium* sp., *Gluconacetobacter* sp. *Anabaenopsis* sp. and *Azospirillum*sp. All endophetic bacteria produced β 1,3gluconase and indole acetic acid (IAA), while *Pseudomonas* sp. produced salicylic acid and phenylalanine ammonia lyase, *Anabaenopsis* sp. produced phenylalanine ammonia lyase, *Gluconacetobacter* sp. produced chitinase and *Herbaspirillium* sp. produced siderophore. Meanwhile, isolates of *Anabaenopsis* sp. and *Pseudomonas* sp. were the most effective to produce β 1,3glucanase and indole acetic acid compared with other entophytic bacteria.

Key words: Endophytic bacteria, Antagonistic effect, Chitinase, β 1,3 glucanase, Siderophores, Indole acetic acid (IAA), Salicylic acid (SA) and phenylalanine ammonia lyase.

INTRODUCTION

Sugarcane crop affected by many fungal diseases in Egypt where the most destructive is smut disease, causes by the fungus *Sporisoriums citamineum* (Syd.) M. Piepenbr., M. Stoll & Oberw, formerly called *Ustilago scitaminea* **(Stoll et al 2003).** Significant loss in yield and quality was recorded in sugarcane due to smut **(Ferreira and Omstock, 1989; Hoy et al 1986 and Magarey and Croft, 1998).** If smut-infected plant crop is allowed for ratoon, then the magnitude of disease increases. A suceptible variety, the smut incidence increases tenfold from plant crop to first ratoon crop **(James, 1974). Kloepper et al (1992)** called bacteria found within tissues internal to epidermis endophytes. Because quiescent endophytic bacteria can become pathogenic under certain conditions and within different host genotypes **(Misaghi and Donndeling, 1990). James**

and Olivares (1997) adjusted the definition and stated that all bacteria that colonize the interior of plants, including active and latent pathogens, can be considered to be endophytes. Once inside the plant tissue, endophytic bacteria remain localized in specific plant tissue, such as the root cortex or colonize the plant systematically by transport or active migration through the conducting elements or the apoplast (**James et al 1994 and Mahaffee and Kloepper, 1997)**. Endophytic bacteria have been isolated from both monocotyledonous and dicotyledonous, ranging from woody tree species, such as Oar **(Brooks et al 1994**) and Pear **(Whitesides and Spotts, 1991)**,to herbaceous crop plants, such as sugar beets **(Jacobs et al 1985**) and maize (**Mcinroy and Kloepper, 1995 and Gutierrez-Zamora and Martinez-Romer, 2001).**The variation in bacteria that has been reported as endophytes spans a significant range of Gram-positive and Gram-negative bacteria or members of the group of the pink-pigmented facultatively methylotrophic bacteria **(Kobayashi and Palumbo, 2000 and Lodewyckx et al 2002a).** Bacterial endophytes have been shown to prevent disease development through endophyte-mediated de novo synthesis of novel compounds and antifungal metabolites **(Strobel et al 2004)**.The ability of some endophytes to show resistance to heavy metals /antimicrobials and degrade organic compounds probably stems from their exposure to diverse compounds in the plant / soil riche.This natural ability to degrade these xenobiotics is being investigated with regard to improving phytoremediation **(Barac et al 2004, Porteouas-Moor et al 2006 and Ryan et al 2007).**The beneficial effects of bacterial endophytes on their host plant appear to occur through similar mechanisms described for plant growth-promoting rhizobacteria (PGPR) according to **Kloepper et al (1991) and Höflich et al (1994).**This makes sense because most of the bacterial endophytes isolated from trees, healthy crops and weeds can be considered to be facultatively endophyte and are capable of living outside plant tissues as rhizospheric bacteria **(Difior and Del Galla, 1995**). Certain endophytic bacteria trigger a phenomenon known as induced systemic resistance (ISR), which is phenotypically similar to systemic acquired resistance (SAR).SAR development when plant successfully activate their defense mechanism in response to primary infection by a pathogen, notably when the latter induces a hypersensitive reaction through which it becomes limited in a local necrotic lesion of brown desiccated tissue **(Van Loon et al 1998).**

The aim of this study was to isolate endophytic bacteria from sugarcane stalks collected from different regions in Upper Egypt and examining its antimicrobial activity against *Sporisorium scitamineum, in vitro*. Characterization of the most potent antagonistic isolates and analyzing its ability to produce certain secondary metabolits which may responsible for pathogen suppression (chitinase, β-1, 3 glucanase, siderophores, salicylic acid, phenylalanine ammonia lyase and indole acetic acid).

MATERIAL AND METHODS

Isolation and purification of endophytic bacteria from sugarcane plants

Sugarcane stalk samples (160 samples) were collected from five governorates (El-Giza, Beni-Suef, Sohag, Qeina and Luxor) during growing seasons 2013-2016. Collected samples were washed with sterile distilled water to remove the soil and subjected to surface sterilization in laminar air flow chamber. These sample were disinfected superficially according to **Araujo et al (2002) and Queiroz et al (2012),** as the following protocol : the hard rind (skin) of the cane was removed using sterile knife, rinsed in 70% ethanol for 3 min and then washed with sterile water for three times. The second-stage surface sterilization was done with 30% hydrogen peroxid for 3 min and then washed with sterile water for three times. The surface sterilized internode portions were cut into pieces, subjects to maceration in sterile condition and macerated sap was used for isolation of endophytic bacteria. The cut samples were ground with 90 ml of aqueous solution (0.9%Nacl) using a sterile mortar and pestle. The tissue extract was subsequently incubation at 28°C for 3hours to allow the complete release of endophytic bacteria from the host tissue. The tissue extract was diluted (10^{-1} and 10^{-2}) in aqueous solution (0.9%Nacl). Five-hundred microlitter/ dilution was cultivated on plates containing LGI medium (K_2HPO_4 0,2g, KH2PO4 0.6g, $MgSo_4$ + $7H_2O$ 0.2g, Na_2 MOo_4 + $2H_2O$ 0.002g, $CaCl_2$+ $2H_2O$ 0.02g, Sucrose 100g, Bromothymol blue 5 ml, Ph=5.5-6) and spread using sterilized L–

shaped glass rod. Inoculated plates were incubated for up to 15 days at 30^{+}2°C. Different types of bacterial colonies were selected on days 5, 10 and 15 days of incubation depending on the morphological characteristic (color, size and shape) and their time of growth. Selected bacteria were picked up and streaked on petri-dishes containing an appropriate medium for purification purified bacterial isolates were transferred to slants of GYC medium (yeast extract 5g ;glucose 15g and 0.1 % chloramphenicol) amend with / without 0.5 g /l active charcoal and incubated at 30 °C for 48-72 h.) and kept at 4°C for further investigation or in 20%sterilized glycerol at -80°C for long preservation.

Antagonistic activity of endophytic bacteria against growth of *S. scitamineum, in vitro*

Screening for assessing antagonistic property of endophytic bacteria against *S. scitamineum* was carried out by dual culture technique according to **Maurhofer et al *(*1995)**. Disk (5mm)of freshly cultured fungus grown on potato dextrose agar (PDA) medium was taken, and inoculated on one side of each Petri plate containing PDA medium and incubated for 7 days at 28 C. After ensuring minimum growth of *S. scitamineum*, the endophytic bacteria were streaked on the other side of the PDA medium and plates were incubated at 28 C for another 15 days. The Petri plates inoculated with fungus alone served as control. Both the pathogen and endophytic bacteria were allowed to grow, and reduction of *S. scitamineum* mycelia growth by endophytic bacteria was calculated. The experiments were conducted in three replicate plates. The percentage of mycelia growth reduction (PMGR) was calculated as follows:

$$X = 100- [G_2 / G_1 x100]$$

Where: X: % of reduction in growth.
G_1: growth of pathogenic fungus in control plates.
G_2: growth of pathogenic fungus in dual plates with *bacteria*.

Identification of endophytic bacteria

Morphological and biochemical characters of the selected 10 bacterial isolates were made according to the methods described by **Fahy and Persly (1983), Lelliott and Stead (1987), Schaad (1988) and Holt et al (1994)**.The developed colonies were examined macroscopically(shape of bacterial cells, sporulation and reaction to gram stain). Bacteriological characteristics were included oxidase, levan production, arginine hydrolysis, gelatin liquefaction, starch hydrolysis and utilization from maltose, glycerol, arabinose, sorbitol, mannitol, xylose and glucose.

Production of secondary metabolites

1. Salicylic acid (SA)

The ten selected endophytic bacterial isolates were grown at 35±2°C for 5 days on a rotary shaker incubator in 250 ml conical flasks containing 50 ml of succinate medium (succinic acid,4.0 g; K2HPO4,6.0 g; KH_2PO_4,3.0g; (NH2) SO_4 ,1.0 g; $MgSO_4$-$7H_2O$ 0.2 g; distilled water. 1000ml; pH 7.0)**.** The cultures were then centrifuged at 10000 rpm for 10minand 4ml of the filtrate was acidified with 1N HCl to pH 2.0, then SA was extracted in equal volume of $CHCl_3$. To the pooled $CHCl_3$ phases, 4ml of distilled water and 5ml of 2M $FeCl_3$ were added. The absorbance of the purple iron–SA complex, which was developed in the aqueous phase was read at 527nm using Milton Roy Spectronic1201 Spectrophotometer. A standard curve was prepared with SA dissolved in succinate medium. The quantity of SA in the culture filtrate was expressed as mg/ml **(Meyer et al 1992**).

2. Indole acetic acid (IAA)

To determine the ability of the tested endophytic bacteria to produce IAA, a colorimetric technique was performed using the Van UrkSalkowski reagent (1 ml of 0.5 M FeCl3 and 50 ml of 35 %HClO4 in water); the microorganisms were grown in nutrient broth containing 0.2% L-tryptophan and incubated at a 35ºC of temperature for 5days. After the incubation time cultures were centrifugated (1000 rpm) then 1 ml of the supernatant mixed with 2 ml of the reagent and incubated for 25 min. at room temperature. The optical density was measured using the wavelength 530 nm. A standard curve of pure IAA (Sigma-Aldrich) was used as standard to calculated IAA production from tested isolates **(Bricet et al 1991).**

3. Siderophore

The tested endophytic bacterial isolates were grown in KB broth for 5 days at 35±2°Cand centrifuged at 10000 rpm for 10min. The pH of the supernatant was adjusted to 2.0 with diluted HCl and equal quantity of ethyl acetate was added in a separating funnel, mixed well and ethyl acetate fraction was collected. Five milliliters of ethyl acetate fraction were mixed with 5ml of Hathway's reagent (1.0 ml of 0.1M $FeCl_3$ in 0.1 NHCl to 100 ml distilled waterthen 1.0 ml of potassium ferricyanide). The absorbance for dihydroxy phenols was read at 700 nm using Milton Roy Spectronic 1201Spectrophotometer (**Reeves et al 1983).**

4. Phenylalanine ammonia lyase (PAL)

Bacterial isolates were grown in 250 ml conical flasks containing 50 ml of peptone medium contained phenol (0.2%), incubated at 30°C for 5 days on a rotary shaker incubator.The cultures were centrifuged at 10000 rpm for 25 min at 4^oC.The supernatant was used for enzyme assay immediately. phenylalanine ammonia lyase (PAL) activity measured using method of **Green et al (1975)**. Absorbance was measured at 620 nm using Milton Ray spectronic 1201 Spectrophotometer.

5. β-1, 3 glucanase

Bacterial isolates were grown in 250 ml conical flasks containing 50 ml of peptone medium contained laminarin (0.2%) (From *Laminaria digitate* Sigma-Aldrich) **Lim et al (1991)**, and incubated at 35°Cfor 5 days on a rotary shaker incubator. The cultures were then centrifuged at 8000 g for 25 min at $4^oC$and the resulted supernatant was used as a crude enzyme source. The reaction mixture, the substrate laminarin (2.5% w/v) in 10mM ammonium acetate, pH 6.0, and 1 mM DTT, was incubated at room temperature for 24h. Samples were assayed for the release of reducing sugars according to the Somogyi-Nelson method **(Nelson, 1944)** modified by **Naguib, (1965)**. Absorbance was measured at 660 nm using a Milton Roy Spectronic 1201 Spectrophotometer. Standard curve of glucose was used as reference **(Lim et al 1991).** B 1,3 glucanase activity was determined as µg of glucose released / ml /min.

6. Chitinase

Bacterial isolates were grown in 250 ml conical flasks containing 50 ml of chitin–peptone medium for bacterial isolates (glucose 0.5%, peptone 0.2%, colloidal chitin 0.2% prepared from crab shell chitin (Sigma) according to **Berger and Reynolds (1958)**, (K_2HPO_4 0.1%, $MgSO_4$-$7H_2O$ 0.05% and NaCl 0.05%, pH 6.8) (**Lim et al 1991)**. The bacterial cultures were incubated at 35°Cfor 5 days. After the incubation period, the cultures were centrifuged at 10000 rpm for 20 min. at 4°C and the supernatant was used as crude enzyme source. A mixture of crude enzyme source (1 ml) and suspension of colloidal chitin (1 ml; 0.1% in 50 mM sodium acetate buffer; pH 5) was incubated at 38°C in a water bath with constant shaking. After 2hr, the release of N-acetylglucosamine in the reaction mixture was estimated by the method of **Reissig et al (1955)**. The enzyme activity was determined using N-acetylglucosamine (Sigma) as a standard. Absorbance was measured at 660nm using a Milton Roy Spectronic 1201Spectrophotometer. One unit of chitinase is defined as the amount of enzyme producing 1 µmolN-acetylglucosamine/min in 1 ml of reaction mixture under standard assay conditions. Specific activity was expressed as µg of glucose released / ml /min.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) with SPSS software (version 8). The treatment means were separated by the least significant difference (LSD) test at $P < 0.05$. (**Snedecore and Cochron, 1982)**

RESULT AND DISCUSSION

Isolation of endophytic bacteria

A Total of 240 bacterial isolates were isolated and established in pure cultures from 160 sugarcane stalk samples, collected during seasons 2013-2016 from five governments in Egypt **(Table 1).**

Table 1. Endophytic bacteria isolated from sugarcane stalk samples which collected from different governments, during seasons 2013-2016

Government	No. samples	No. isolates
Giza	25	30
Sohag	21	40
Luxor	44	60
Qena	50	70
Bani-Suef	20	40
Total	160	240

Antagonistic effect of endophytic bacteria against *Sporisorium scitamineum*, *in vitro*

Data in **Table (2)** summarize the results of the *in vitro* assay by which 240 bacterial isolates were evaluated for their antagonistic effect against *Sporisorium scitamineum*, on PDA medium. Only, 62 isolates caused moderate to strong inhibition to the pathogen on PDA plates.

Table 2. Antagonistic effect of selected sugarcane endophytic bacterial isolates on the growth of *Sporisorium scitamineum*, on PDA medium using dual culture assay*

Isolate code	Mycelial growth reduction (%)*	Isolate code	Mycelial growth reduction (%)	Isolate code	Mycelial growth reduction (%)
Mex-58-1866	14.1	LU22	9.6	LU44	44.4
Mex-2001-80	48.9	LU23	24.2	LU45	41.2
Sp-81-1763	7.1	LU24	23.0	LU46	26.7
C9	26.7	LU25	6.5	LU47	19.1
Sp-80-1842	7.7	LU26	21.0	LU48	5.9
Sp-79-2233	28.0	LU27	23.5	LU49	29.4
Sp-70-1143	6.6	SO28	29.4	LU50	26.7
Sp-59-56	19.1	SO29	39.9	LU51	7.0
P80-3280	14.7	SO30	6.9	LU25	19.1
Mex-58-1868	6.5	SO31	5.4	Q53	8.9
Sp-27-5181	29.4	BAN32	20.4	Q54	8.4
C9	13.4	BAN33	54.7	Q55	17.2
C9	7.7	BAN34	58.4	Q56	41.8
Q14	23.0	BAN35	15.9	Q57	6.6
Q15	21.7	BAN36	7.7	Q58	6.5
Q16	29.8	BAN37	14.7	Q59	20.4
Q17	44.4	BAN38	9.0	Q60	5.1
Q18	26.7	BAN39	11.5	Q61	6.4
Q19	42.5	LU40	5.3	Q62	49.4
Q24	45.3	LU41	25.7	Q63	7.0
LU20	26.7	LU42	14.1		
LU21	7.7	LU43	25.7		

*Mycelial growth reduction %, in dual plates, compared to pathogen control.

The results indicated that percentage of mycelia growth reduction was mainly ranged from 5.1% to 58.4 %. The ten most antagonistic bacterial isolates (Mex-2001-80; Q17; Q24; SO29; BAN33; BAN34; LU44; LU45, Q56 and Q62) were selected for further studies, where percentage of mycelia growth reduction were 48.9, 44.4, 45.3, 39.9, 54.7, 58.4, 44.4., 41.2, 41.8 and 49.4%, respectively**.**

Identification of antagonistic endophytic bacteria

Ten isolates of endophytic bacteria were selected, based on their antagonistic efficacy against smut fungus, *Sporisorium scitamineum*, *in vitro* and identified. Data in **Tables (3 and 4)** revealed that four isolates (BAN33, BAN34, Q56 and Q62) were short-rod, negative-gram and non-sporulation, and the others six isolates were long-rod, positive–gram and sporulation. Meanwhile, isolates BAN33 and BAN34 were positive with oxidase, arginine hydrolysis, starch hydrolysis and utilization of glycerol, arabinose, mannitol, xylose and glucose and were negative with levan production, gelatin liquefaction and utilization of maltose and sorbitol.But,Q56 isolate was negative with oxidase, levan production, arginine hydrolysis, gelatin liquefaction starch hydrolysis and utilization of maltose, glycerol and sorbitol, and was positive with utilization of arabinose, mannitol, xylose and glucose, but Q62 isolate was positive with oxidase, gelatin liquefaction, starch hydrolysis and utilization of maltose, sorbitol, arabinose, mannitol, xylose and glucose, and was negative with levan production, arginine hydrolysis, and utilization of glycerol. However, isolates SO29 was positive with arginine hydrolysis, gelatin liquefaction and utilization of maltose, sorbitol, arabinose, mannitol, xylose and glucose and was negative with oxidase, levan production, starch hydrolysis and utilization of glycerol. Also, isolate Mex-2001-80 was positive with oxidase, levan production, arginine hydrolysis, gelatin liquefaction, starch hydrolysis and utilization sorbitol and mannitol, and was negative with utilization of maltose, glycerol, arabinose, xylose and glucose. Meantime, isolates Q17 and Q24 were positive with oxidase, arginine hydrolysis, gelatin liquefication, and utilization of maltose, sorbitol, arabinose, mannitol, xylose and glucose and were negative with levan production, starch hydrolysis

Table 3. Morphological and biochemical characteristics of endophytic bacteria isolated from sugarcane plants.

Isolate	Reaction							
	Gram stain	Cell shape	Spore formation	Oxidase	Levan production	Arginine hydrolysis	Gelatin liquefaction	Starch hydrolysis
Mex-2001-80	+	Long rod	+	+	+	+	+	+
Q 17	+	Long rod	+	+	-	+	+	-
Q 24	+	Long rod	+	+	-	+	+	-
SO 29	+	Long rod	+	-	-	+	+	-
BAN 33	-	Short rod	-	+	-	+	-	+
BAN 34	-	Short rod	-	+	-	+	-	+
LU 44	+	Long rod	+	+	-	-	+	+
LU\ ARM 45	+	Long rod	+	+	-	-	+	+
Q 56	-	Short rod	-	-	-	-	-	-
Q 62	-	Short rod	-	+	-	-	+	+

Table 4. Utilization of different carbon compounds by endophytic bacterial isolates, obtained from sugarcane plants.

Isolate	Reaction with						
	Maltose	glycerol	sorbitol	arabinose	mannitol	xylose	Glucose
Mex-2001-80	-	-	+	-	+	-	-
Q 17	+	-	+	+	+	+	+
Q 24	+	-	+	+	+	+	+
SO 29	+	-	+	+	+	+	+
BAN 33	-	+	-	+	+	+	+
BAN 34	-	+	-	+	+	+	+
LU 44	-	+	+	+	+	+	+
LU \ ARM 45	-	+	+	+	+	+	+
Q 56	-	-	-	+	+	+	+
Q 62	+	-	+	+	+	+	+

and utilization of glycerol,but isolates of LU24 and Lu/ Arm45 were positive with oxidase, gelatin liquefaction, starch hydrolysis and utilization of sorbitol, glycerol, arabinose, mannitol, xylose and glucose and were negative levan production, arginine hydrolysis and utilization maltose.

According to previously described morphological and physiological characteristics of **Bergey's Manualof Determinative Bacteriology,** isolates BAN33 and BAN34 identified as *Pseudomonas* sp., isolate Q56 as *Serratia* sp., isolate Q62 as *Entrobacteria* sp., isolate SO29 as *Herbaspicillum* sp. , isolate MEX-2001-80 as *Gluconacetobacter* sp., isolates LU44 and LU/ARM45 as *Azospirillum* sp., and isolates Q17 and Q24 as *Anabaenopsis* sp.

Numerous plant growth promoting bacteria of the genera *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Azospirillum*, *Klebsiella*, and *Enterobacter* have been isolated from the rhizosphere and phyllosphere of various crops and noted for their synergistic effects on plant growth **(Kloepper and Beauchamp 1992; Egamberdiyeva and Höflich, 2001**

and Egamberdieva, 2008). Endophytic bacteria have been isolated from all plant compartment including seeds (**Posada and Vega, 2005**). **Lodewycrx et al (2002b)** highlights the methods used to isolate and characterize endophytic bacteria from different plant species. Avery comprehensive list of bacterial endophytes isolated from a board range of plants was provided by **Rosenbleth and Martinez-Romero (2006).** Endophytic bacteria can be isolated from surface-disinfected plant tissue or extracted from inside the plant and if it does not visibly harm the plant (**Hallman et al 1997**) to colonize the internal plant tissues, it has been proposed that bacterial endophytes gave genomic differences compared to rhizosphere colonizing bacteria, although so far no definitive group of genes have been identified that is responsible roles in endophytic behavior was identified by **Ali et al (2014)** by comparing the complete genomes of nine bacterial endophytes. **Kloepper and Beauchamp (1992)** attempts to evaluate total populations of bacteria in plant may produce varied result, depending on the growth media used for isolation, variations in the growth conditions of the host plant, and the way the plant tissue was used.

Production of secondary metabolites which responsible for antimicrobial activities

Results in **Table (5)** show that selected endophytic bacterial isolates were significantly differed in producing salicylic acid, IAA and siderophores. *Anabaenopsis* sp. Isolates showed the highest significant ability in producing IAA (1.240 mg/ml/30min) followed by isolate *Pseudomonas* sp., *Gluconacetobacter* sp., *Herbaspicillum* sp., *Anabaenopsis* sp., *Entrobacteria* sp., *Azospirillum* sp., *Serratia* sp.and *Azospirillum* sp.respectively.All tested bacterial isolates did not produce sidrophores in the medium except *Anabaenopsis* sp. Isolate. None of the selected bacterial isolates were able to produce salicylic acid in the medium except isolate *Pseudomonas* sp. which produced 0.262-0.316 mg/ml. These results agree with results of **Montealegro et al (2003).**The increase in plant growth might be associated with secretion of auxins, gibberellins and cytokinin **(Dubeikovsky et al 1993)** and suppression of deleterious microorganisms in the rhizosphere (**Gamliel and Katan, 1993).** On the other hand, siderophores are low weight compounds with high affinity for Fe+3 **(Neilands, 1981),** which are produced under limiting concentration of iron. These compounds can transport this element inside the cell for metabolic functions **(Press *et al.,* 2001),** and microorganism which are able to produce siderophores show competitive advantage as compared to those that do not produce them. From this point of view, the competence for iron increases in conditions where this element is limiting, but this condition is reverted when iron is added to the culture medium **(Elad and Baker, 1985)**.

Table 5. Estimation of indole acetic acid (IAA), salicylic acid (SA) and siderophores content produced by different endophytic bacteria from sugarcane plants

Isolate	Code	Salicylic acid (mg/ml)	IAA (mg/ml/ 30min)	Siderophore (adsorption at 700 nm)
Gluconacetobacter sp.	Mex-2001-80	0.0	0.351367	0.0
Anabaenopsis sp.	Q 17	0.0	0.2794	0.0
	Q 24	0.0	1.240367	0.0
Herbaspicillum sp.	SO 29	0.0	0.342053	0.11
Pseudomonas sp.	BAN 33	0.262167	0.960967	0.0
	BAN 34	0.316333	0.4699	0.0
Azospirillum sp.	LU 44	0.0	0.1397	0.0
	LU45	0.0	0.160867	0.0
Serratia sp.	Q 56	0.0	0.15875	0.0
Entrobacteria sp.	Q 62	0.0	0.2413	0.0
LSD at 5%		0.12	0.25	0.01

Plant growth-promoting bacteria (PGPR) may affect plant growth either directly or indirectly. Direct promotion of plant growth occurs when either (1): The PGBR facilitates the acquisition of resources from the environment including nitrogen, phosphorous and iron or (2): modulates plant growth by providing or regulating various plant hormones including auxin, cytokinin or ethylene. Indirect promotion of plant growth by PGBR occurs when a bacterium limits or prevents the damage to plants that might otherwise be caused by various pathogenic agents including bacteria,

fungi and nematodes. There are many common mechanisms that PGBR use to indirect promote plant growth including the production of antibiotics, cell wall-degrading enzymes, lowering plant ethylene levels, induced systemic resistance, decreasing the amount of iron available to pathogens and the synthesis of pathogen-inhibiting volatile compounds **(Glick, 2015).**

Data in **Table (6)** showed that all the bacterial isolates were unable hydrolyze Chitin *in vitro* except isolate *Gluconacetobacter* sp. which showed a chitinase activity of 0.003g of glucose released /ml /hr. all the bacterial isolates were able hydrolyze β-1, 3 glucanase, *in vitro* isolate *Anabaenopsis* sp., *Pseudomonas* sp., *Azospirillum* sp., *Gluconacetobacter* sp., *Anabaenopsis* sp., *Entrobacteria* sp., *Serratia* sp., *Azospirillum* sp. and *Herbaspicillum* sp. which showed β-1, 3 glucanase activity of 0.0033 -0.0031 -0.002– 0.0015-0.001-0.0009 -0.0007 -0.0002 -0.0002 g of glucose released /ml /15min, respectively. Only isolates of *Anabaenopsis* sp. and *Pseudomonas* sp. produced phenylalanine ammonia lyase (PAL) compared with other isolates.

Table 6. Activity of chitinase, β-1,3-glucanase and phenylalanine ammonia lyase (PAL) enzymes of different endophytic bacteria, isolated from sugarcane plant.

Isolate	code	PAL	Glucanase ug/ml/15min	Chitinase ug/hr/ml
Gluconacetobacter sp	Mex-2001-80	-	1.0	3.4
Anabaenopsis sp.	Q 17	+	1.0	0.0
	Q 24	+	3.0	0.0
Herbaspicillum sp.	SO 29	-	0. 16	0.0
Pseudomonas sp.	BAN 33	+	3.0	0.0
	BAN 34	+	2.0	0.0
Azospirillum sp.	LU 44	-	0.207	0.0
	LU45	-	1.5	0.0
Serratia sp.	Q 56	-	0.728	0.0
Entrobacteria sp.	Q 62	-	0.934	0.0
LSD at 5%			0.99	0.03

Aktuganov et al (2003) reported that of 70 tested *Bacillus* spp. strains antagonistic to phytopathogenic fungi, 19 were found to possess chitinolytic activity when grown in liquid cultures. These results in agreement with those found by **Aktuganov et al** (**2008)** in a study on 18 strains of *Bacillus subtilis* and 9 strains of *Paenibacillus ehimensis* revealed that β-1,3-glucanase was more significant factor than chitinase for determining the mycolytic potential of bacteria and their ability to utilize the mycelium of phytopathogenic fungi as a growth substrate. **Santoyo et al (2016)** reported that bacterial endophytes with high locally induced 1-aminocyclopropane-1-carboxylase (ACC) deaminase activities might be excellent plant growth-promoters, because they ameliorate plant stress by efficiently blocking ethylene production.

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البكتريا الداخلية المصاحبة لقصب السكر وقدرتها على تثبيط فطر *Sporisorium scitamineum* مسبب مرض تفحم قصب السكر

[74]

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الموجـــــز

أجريت هذه الدراسة بغرض عزل وتعريف البكتريا الداخليه المستوطنة لسيقان نباتات قصب السكر، ودراسة كفاءتها فى تثبيط الفطر *Sporisorium scitamineum* المسبب لمرض تفحم قصب السكر وكذلك انتاج بعض الانزيمات ومنظمات النمو .جمعت 160 عينة لسيقان قصب السكر (صنف –GT–54) 9 من خمس محافظات بجمهورية مصر العربية (الجيزه – بنى سويف – سوهاج – قنا – الاقصر) خلال مواسم 2013–2016، عزل منها 240 عزله بكتيريه. تم أختبار النشاط التضادى للعزلات البكتيرية ضد الفطر *S. scitamineum* فى المعمل، وذلك باستخدام طريقة أطباق الأجار المزدوجة حيث أظهرت 62 عزله فقط كفاءه تضاديه للفطر الممرض، وتم انتقاء أفضل عشره عزلات بكتيريه تضاديه لدراسه كفاءتها لانتاج انزيمات فينيل ألانين أمونيا لايز ، بيتا 1،3 جلوكانيز والكيتينيز بالاضافةإلى انتاج حمض السالسيليك، واندول حمض الخليك، وحاملات الحديد. أوضحت اختبارات تعريف العزلات المنتقاة بناء على الصفات المورفولوحيه والفسيولوحيه بالمطابقه مع Bergy's manual أن تلك العزلات تتبع انواع الأجناس:

Pseudomonas sp. *Herbaspirillium* sp., *Gluconacetobacter*sp. sp., *Anabaenopsis* sp. and *Azospirillum* sp., *Serratia* sp., *Entrobacteria* sp.,

اظهرت جميع العزلات المختبره كفاءه لانتاج كلا من بيتا 1،3 جلوكانيزواندول حمض الخليك، بينما انتجت عزلة بكتريا *Pseudomonas* sp., انزيمات فينيل الانين امونيا لاييزوحمض السالسيليك، أماعزله بكتريا *Anabaenopsis* sp. انتجت فينيل الانين امونيا لاييز، بينما انتجت عزله بكتريا *Gluconacetobacter* sp. انزيم الكيتينيز وانتجت عزله بكتريا *Herbaspirillium* sp. حاملات حديد. أماعزلات *Pseudomonas sp.* و *Anabaenopsis* sp. كانت الاكثر كفاءه لانتاج اندول حمض الخليك وانزيم بيتا 1،3 جلوكانيز

الكلمات الدالة: البكتريا الداخلية، تأثير التضاد، إنزيم الكيتينيز، إنزيم بيتا1–3، جليكونيز، سيدروفورز، إندول أسيتيك أسيد، ساليليك أسيد، فينيل آلانين

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