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Antifungal Metabolites of Suppressive Strains of Root-Infecting Diseases of Sugar Beet Pseudomonas Biocontrol

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Current study dealt with five of rhizospheric *fluorescent pseudomonad* strains previously found to have the potential to reduce sugar beet root-infecting *Fusarium oxysporum* f. sp. *Betae* in greenhouse. They were analyzed for their mode of action of biocontrol activity. It was found that they differed in their potency to produce secondary metabolites that can influence the pathogen by direct or indirect effects. *Pseudomonas putida* strain PS23 was shown to be superior in the antibiosis and activity to produce chitinase and cellulase enzymes as well comparable to the other strains. Antifungal metabolites produced by this bacterial strain were characterized by GC/MS. Several chemical constituents could be detected, where phenolic compounds were abundantly characterized. Plenty of compound types such as monoterpenoides, phenylpropenes, diterpenoides known as pest control agents were detected along with other several allelochemicals existed in metabolic products of this efficient rhizobacterial biocontrolagent.

Keywords: Biocontrol agent, *Fusarium oxysoorum*f.sp, *betae* and Rhizospheric fluorescent pseudomonads.

Sugar beet (*Beta vulgaris* L.) is grown in Northern and southern Egypt, producing approximately 10,000,000 tons. Sugar productivity is affected by plant infection with some serious diseases as reported by Whindels *et al.* (2005). *Fusarium oxysporum* f.sp. *betae* is the most common fungal root pathogen in beet grown fields worldwide.

As increasing use inputs in agriculture have caused bad effect on environment and man health, thus biological control is being considered as alternative clean method to the benefit of crop production (Welbaum *et al.*, 2004).

Several investigators suggested the use of fluorescent pseudomonads as seed or soil treatment to control the major soil-borne diseases to decrease the bad effect of pesticides on the surrounding environment (El-Assiuty *et al.*, 2010b; Hammami *et*

al., 2013). Fluorescent pseudomonads are heterogeneous group of rhizobacteria, embracing some Non-pathogenic species that colonize the root surfaces and rhizosphere (Kloepper *et al.,* 1980).

In a recent investigation, five efficient rhizospheric strains of fluorescent Pseudomonads having the potency to manage root-infecting *F.oxysporum* f.sp. *betae* in sugar beet were identified and suggested to be used in controlling root-infecting diseases of sugar beet (Attia *et al.,* 2016). Mode of action of fluorescent pseudomonads in biological control of plant diseases was intensively studied by several investigators. Mechanisms of biological control of plant pathogens by fluorescent pseudomonads involve competition for nutrients, production of secondary metabolites such as iron chelating siderophores, hydrogen cyanide (HCN), antibiotics, extracellular lytic enzymes (Van Loon *et al.,* 1998). Synergistic combination was reported for successful antifungal interaction by some investigators (O'Sullivan and O'Gara, 1992).

Characterization and identifying of antifungal metabolites produced by these five efficient *Pseudomonas* strains was undertaken in the present study.

Materialsand Methods

1. Synthesis of allelochemicals:

1.1. Production of extracellular hydrolytic enzymes:

1.1.a. Production of cellulase:

Method adopted by Carder (1986) was followed. The five strains of *Pseudomomas* spp. were grown on cellulose agar (per 1L, yeast extract 1g, CMC 10g , KH_2PO_4 4g, NaCl 2g, $MgSO_4.7H_2O$ 1g, Mn SO_4 0.05g, $FeSO_4.7H_2O$ 0.05g, $CaCl_2.2H_2O$ 2g, NH_4Cl 2g. pH7.0–7.4) and plates were incubated at 28°C for 4 days. Thereafter, plates were flooded with 0.1% Congo red, left for 15-20 min, washed with 1ml NaCl (1M) and incubated in refrigerator at 8°C over night. Appearance of clear zones around colonies was recorded as positive reaction for producing cellulase.

1.1.b. Lipase activity:

Bacterial cultures were grown on nutrient agar amended with egg yolk (Omidvari, 2008). After 24 hr of incubation, clear zones around the colony indicates positive for lesithinase activity. The plates were flooded with saturated CuSO4 solution and dried at 37°C for 20min. Appearance of blue greenish color on the surface around the colony indicates lipase activity (Cowan, 1974).

1.1.c.Pectinase activity:

Bacterial strains were cultured on pectin agar (per 1l, yeast extract 1g, pectin 5g, KH_2PO_4 4g, NaCl 2g, $MgSO_4.7H_2O$ 1g, Mn SO_4 0.05g, $FeSO_4.7H_2O$ 0.05g, $CaCl_2.2H_2O$ 2g, NH_4Cl 2g. pH7.0 –7.4). Plates were, and then incubated at 28°c for 4 days, and then flooded with 0.1% Congo red and left for 15-20 min. Plates were, then washed with 1ml NaCl (1M) and incubated in refrigerator at 8°C overnight. Positive reaction was indicated by clearance of clear zones around colonies (Fogarty and Kelly, 1983).

1.1.d.Gelatinase activity:

Gelantinase activity was performed by inoculating a loop from the bacterial culture into gelatin tubes (peptone 5g, beef extract 3g, gelatin 12.0g pH6.8±0.2) incubated for 4 to 7 days and refrigerated for half an hour. Positive reaction causes the solidification of the liquid medium after refrigeration (Blazevic and Ederer, 1975).

1.1.e.Amylase activity:

Amylase activity was indicated by clear zone around the bacterial colonies on starch agar medium. After 72 to 96 hr of incubation, the plates were flooded with iodine solution for 1min and poured off the excess iodine. The appearance of clear zone surrounding the colony indicates positive result for starch hydrolysis activity (Collins *et al.*, 1995).

1.1.f.Protease activity:

Protease medium was used to determine the protease activity in 7% skim milk. Clear zone around the bacterial colony was recorded as positive activity as described by Chaiharn et *al.*(2008).

1.1.g. Chitinase activity:

Colloidal chitin was prepared from chitin as described by Rodriguez-kabana *et al*. (1983). The bacterial strains were grown on chitin medium for 4 days at 28^0C. Thereafter, plates were incubated in refrigerator at 8°C overnight. Clear zones observed around the bacterial colonies indicated as the utilization of colloidal chitin.

2. *Production of hydrogen cyanide (HCN):*

HCN was determined following the method of Bakker and Skipper (1987). Each of the bacterial strains were inoculated onto liquid King's B medium (KB) supplemented with 4.4 g glycine/L, then sterilized filter paper was soaked in2.0% Na_2CO_3 in 0.5% (w/v) picric acid in a test tube and suspended at one side of the tube. The test tube was sealed with parafilm. Test tubes were incubated for 4 days at

28+2°C. Color change of the filter paper from deep yellow to orange and orange to brown indicates the production of HCN.

3. Production of indole acetic acid (IAA):

Bacterial strains were inoculated in King's Broth (20ml) supplemented with 5g/ 1000ml tryptophan and incubated for 24hr in 28°C on rotary shaker. Cultures were centrifuged at 10.000 rpm for 15min. Two drops of orthophosphoric acid and 4ml Salkowski reagent ($FeCl_3$ 2%, $HClO_4$ 35%) were mixed with the culture supernatant (2ml) and incubated for 25min at room temperature. Development of pink to red colour after 0.5 to 3h indicated IAA production and the quantitative estimation of IAA were performed by using standard graph as described by Patten and Glick (2002).

4. Antibiotic production:

Capacity of the strains to produce antibiotics was performed in the growing culture. An indirect qualitative method based on the detection of biosynthetic genes by PCR was followed for each antibiotic. Primers of antibiotics used in the present study are shown in Table (1).

Table 1. Primers for PCR analysis of the known antibiotics.

Primer	Sequence 5'→ 3'	Expected Products size (bp)	G+C %	Tm** (ºC)	Length	Reference
PhlA-1f PhlA-1r	TCAGATCGAAGCC CTGTACC GATGCTGTTCTTG TCCGAGC	418	60 55	61.4 59.4	20 20	Rezzonico*et al*. 2003)
PHz1 PHz2	GGCGACATGGTCA ACGG CGGCTGGCGGCGT ATAT	1.400	60 55	61.4 59.4	20 20	Delaney *et al*. (2001)
PltBf PltBr	CGGAGCATGGAC CCCCAGC GTGCCCGATATTG GTCTTGACCGAG	700- 900	73.7 56	65.3 66.3	19 25	Mavrodi*et al*. (2001)
Prncf Prncr	CCACAAGCCCGGC CAGGAGC GAGAAGAGCGGG TCGATGAAGCC	720	75 60.9	67.6 66	20 23	Mavrodi*et al*. (2001)

** Tm= melting temperature

5. Detection of antibiotics produced by strains of Pseudomonas spp.:

Primers for different PCR-based screening of genes that encode for antibiotics are detailed in Table (1). Preparation of bacterial templates for detecting antibiotic producing genes was carried out as described by Wang *et al*. (2001) and Rezzonico *et al*. (2003). PCR amplification of gene primers pHz1-2, PrncF-R, and PhIA-If were carried out in 25 µl reaction mixtures containing 2.5µl of lysed bacterial suspension by using kits of gene Jet geNomic DNA purification (thermo). The cycling program for pHz1-2, PhlA-1f-1r, PrncF-Rincluded an initial denaturation at 95°C for 3min followed by 35cycles of 95°C for 1min, 62°C, 52°C,72°C for 1min, and then a final extension at 72°C for 5min. The amplification products were electrophoresed in 1% (w/v) agarose gel with 1XTBE buffer at 80V at room temperature, stained with ethidium bromide and photo graphed under UV light. For PltBF the PCR program consists of an initial denaturation step at 94°C for 2 min., followed by 30 cycles of 94°C for 60 s, 56°C for 45 s, 72°C for 60 s, and a final elongation at 72°C for 10 min

6. Characterization of antifungal metabolites by GC: MASS:

Analysis of GC/MS/MS was done to characterize and identify the allelochemicals produced by the most efficient candidate (s) of *Pseudomonas* sp. under study as described by Duffy and Defago (1999).Bacterial starter was grown into 10ml dilute (1/10) NBY broth in 20 ml screw top vial for 8 to 12 h at 27°C at 140 rpm. Thereafter, a volume of 1000 µl was inoculated in 20ml NBY amended with 1% glucose and 0.7 mM $CuSO_4$, $ZnSO_4$. pH was adjusted to 6.5 - 6.7. Extraction was made after 48 and 120 h of incubation at 27°C. The Broth culture was filtered then the residue was centrifuged at 3000 rpm. After that the supernatant was directly exposed to the vapor of chloroform by putting every 4 small bottles (20ml sized) in conical flask (250ml) containing 25ml of chloroform, then closed with parafilm for 5days the same time to kill all cells according to the technique supplied by *Vidaver et al.* (1972) and modified by El- Bakery,2010.

The analysis was carried out using a GC (Agilent TechNologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m x 0.25mm i.d. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1ml/min. The injector and detector temperatures were 200°C and 250°C, respectively. Volume injected 1µm of the sample. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250°C. and acquisition mass range 50-800.

The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

Results

1. Biocontrol activity mediated by synthesis of allelochemicals:

Results for the secondary metabolites produced by the bacteria under study are shown in Table (2).

Table 2. Lytic enzymes, IAA, HCN and antibiotics produced by *Pseudomonas* strains under study

Strain	Lytic enzyme							IAA	HCN	Antibiotic			
										Presence (+) / absence(-)			
PS6	**Chit***	**Cellu***	**Lipase**	**Amylase**	**Pectin***	**Protease***	**Gelatin***			**2-4DPG***	**Phz***	**pyolut***	**Pyrrolin***
PS15	-	-	+	-	-	+	-	-	+2	-	-	-	-
PS20	+	+	-	+	-	+3	-	-	-	-	-	-	-
PS21	+	-	+	-	-	+	-	-	-	-	-	-	-
PS23	+	+	+	-	-	+	+	+	-	-	-	+	-

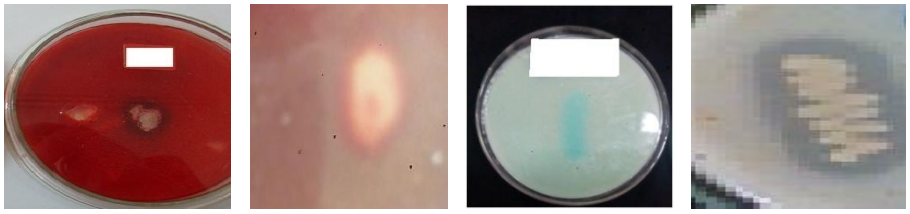
Chit=Chitinase –Cellu= Cellulase – Pectin = Pectinase – protease – gelatin = Gelatinase. Phz =phenazine – pyolut = pyoluteorin – pyrrolin = pyrrolnitrin – 2-4 DPG= 2-4-diacetylphlorogluciNol

2. Production of extracellular enzymes:
The ability of bacterial strains of *Pseudomonas* spp. to produce lytic enzymes (Table, 2).It could be illustrated as follows:

Cellulase: Bacterial growth surrounded by clear halos indicated the positive production of cellulase by *P. fluorescens* strains ns. PS20 and *P. putida* strain No. PS23. While, the other strains could not exhibit the ability to produce cellulase in growing media.

Lipase: Clear zones were observed around the bacterial colonies of P. *putida* strains ns PS15, *P. putida* strain PS21 and *P. putida* strain PS23, indicating their positive lipase activity. Inactivity of lipases, however, was found for *P. aeroginusa* strains No. PS6 and *P. fluorescens* strain PS20.

Pectinase: Appearance of clear halo was not detectable around any of colonies of the bacterial strains indicating the failure to produce pectinase enzyme.
Gelatinase: Gelatinase was detectable (liquefying gelatin), just in test medium of *P. putida* strain No. PS 23.
Amylase: Clear zone was appeared, only around the colony of *P. fluorescens* strain No. PS 20 indicating the positive potential for starch hydrolysis. Whereas, this enzyme was not detectable in media of any of the other three bacterial strains.
Protease: All strains displayed proteolytic activity in skim milk media.
Chitinase: Four out of the five strains of *Pseudomonassp*. under study. Were found to have the potential to produce chitinase in the growing media. *P. putida* strain No. PS15, however, was not able to produce this enzyme.Fig. (1) shows reactions indicating the positive activities of some representative strains in producing the lytic enzymes.



Chitinase Cellulase Lipase Protease

Fig. 1. Production of extracellular enzymes by bacterial strains of *Pseudomonas* spp.

The obtained results indicated that *P. fluorescens* strain No. PS 20 and *P.putida* No. PS 23 efficiently produced both chitinase and cellulase enzymes (the most effective enzymes involved in protecting against the fungal pathogens).

3. Hydrogen cyanide (HCN) production:

Results (Table, 2) show that two strains, namely *P. aeroginosa* and *P. putida* (PS 6 and PS 15, respectively) were shown to have the potential to produce HCN as indicated by changing the color of filter paper.

4. Production of indole acetic acid (IAA):

Results in (Table, 2) indicated that IAA could be produced just by *P. putida* strain No. PS 23, where it could grow in tryptophan-free medium.

*5. Antibiosis***:**

The probability of producing the non-volatile antibiotics could be found only by *P. aeruginosa* strain PS 6 and *P. putida* strain PS 23. It was found that *P. aeruginosa* has the gene of phenazine and *P. putida* involves the gene of pyoluteorin (Table 2).

It could be concluded from the results (Table,2) that *P. putida* strain No. PS 23 ranked the first pseudomonas agent in producing lytic enzymes, IAA and the antibiotic, pyoluteorin comparable with the other *Pseudomonas* strains.

6.GC/MS Characterization of bacterial antifungal metabolites:

Result of the Analyses of culture filtrate of the most efficient strain of P. putida (PS 23) made by GC/MS to assess the common chemical components, Results are presented in Table (3).

Results(Table,3) indicate that GC/MS analyses of culture filtrate of *P.putida* strain PS 23 were contain mostly phenolic compounds. Some other compounds such as scopoletin, salicylic acid, thebaine …and others were found within the culture filtrate of this bacterial strain.

D i s c u s s i o n

The current study dealt with characterizing the mode of action of the secondary metabolites produced by the most efficient *Pseudomonas* strains in controlling *Fusarium oxysporum* f.sp. *betae*. Some of biochemical activity mediated by synthesis of these metabolites could be found throughout executing this study. High activity of lytic enzymes was found in the bacterial filtrates of these strains. Chitinase was efficiently excreted by four out of the five strains under study. Cellulase was also produced by two of these strains. In addition, some of other enzymes, such as lipase, amylase, protease and gelatinase were found to be excreted by some strains. Chitinase and cellulose, as the most important enzymes, were found

to be efficiently produced, in particular by *P.putida* No. PS 23. Chitinase and cellulase are known to play the major role in degrading fungal cell walls as reported by Michell and Alexander (1963). Micro-organisms having the capability of producing chitinases have been shown to be efficient biocontrol agents (Ordentlich *et al*., 1988 and Inbar and Chet, 1991). It is known that the cell walls of *Fusarium* spp. are composed mostly of chitin (47%), with14% glucan (Skujins *et al*., 1965). Similarly, Ordentlich *et al*, (1988) showed that Chitinase was the key enzyme in the dissolution of hyphae of *Sclerotium rolfsii* and the loss of protoplasm in fungal structures and enzymatic dissolution of the cell walls. One of the main mechanisms involved in the antagonistic activity of biocontrol agents is the lysis of propagules that Indole-3-acetic acid (IAA) was produced; only by *P. putida* strain Ps23. IAA has direct or indirect effects on plant pathogens. It has a role in plant root elongation (Arshad and Frankekenberger, 1998) and improving mineral and water uptake (Patten and Glick, 2002). We suggest that the reduction of root-infecting *F.oxysporum* f.sp. *betae* may be attributed to the indirect effect of IAA produced by the bacterial strain Ps23 of *P. putida*. This hypothesis is supported by the finding of Khare and Arora (2010), who reported that charcoal rot of chickpea could be suppressed as an indirect mechanism of *P. aeruginosa*.

The Non-volatile antibiotics, phenazine (PCA) or (Phz) and pyoluteorin (PLT) were found to be produced by only 2 out of the five bacterial strains. These are Ps6 (phenazine production) and Ps23 (pyoluteorin production). Production of antifungal secondary metabolites, such as 2,4-diacetylphloroglucinol, pyoluteorin (PLT), pyrrolnitrin (PRN), phenazines, is a prominent feature of many biocontrol fluorescent pseudomonads (Fenton *et al*., 1992). Antibiosis is implicated, nowadays as an important mechanism of biological control. The rest strains were unable to produce any of the antibiotics as secondary metabolites. This finding gives these two bacterial strains the superiority in using as bioagents to manage root-infecting pathogens. The study revealed that the antibiotic Phz was recovered from the roots and rhizosphere of sugar beet grown from seeds inoculated with phenazine-producing pseudomonads. Also, Leeman *et al.* (1996) reported that antibiotics and siderophores may act as inducers for local and systemic host resistance. Accordingly, the high activity of the two efficient strains of *P. putida* and *P. aeruginosa* in managing the root-infecting pathogen in greenhouse (Attia *et al*., 2016) may be attributed to their ability to produce these antibiotics.

Table 3. Analysis of chemical compounds in culture filtrate of *P. putida* strain No. PS 23

No	Name
1	HydroquiNone
2	4-Methylcatechol
3	Epinephrine
4	4-Hydroxybenzoic acid\
5	2-(2-Hydroxyethoxy)phenol
6	PheNol, 4-(2-arnirropropyl)-
7	Pyrocatechol 3,5-di-tert-butyl-
8	2,5.diamiNo-4,6-dydroxypyrimidine ‘
9	4-Methoxycinçamic acid
10	2,3,5,5,8a-Pentamethy-6,7,8,8a-tetrahydro-5H-chromen-8-ol
11	Sinapic acid
12	Scopoletin
13	5β,7βH,1Oα-Eudesm-11-en-1α-ol
14	2,6-Dihydroxybenzoic acid
15	2,5-di-tert-butyl-p-quiNone
16	Vanillic acid
17	4',6-Dimethoxyaurone trails-
18	7-Methoxy-3-(p-methoxyphenyl)-4H-chrorneri-4-one
19	Mandelic acid, 3,4-dihydroxy
20	Papaveroline
21	4’,7-Dimethoxyisoflavone
22	Phytol
23	Thebaine
24	Camphor
25	4-MercaptopheNol
26	Propyl gallate
27	Nordihydroguaiaretic acid
28	NeoheptaNol
29	4-(Methylthio)benzoic acid
30	Levallorphan
31	Probucol
32	Gentisicacid
33	Thymolphthaein
34	3,5-Di-tert-butyl-4-hydroxybenzoic acid

Hydrogen cayanide (HCN), the volatile antibiotic was found to be produced by two out of the five bacterial strains (Ps6 &Ps15). Production of HCN by certain strains of *Pseudomonas fluorescens* has been involved in suppression of soil borne pathogens (Voisard *et al.,* 1989). Several scientists demonstrated the role of HCN in disease suppression in various crops (Stutz *et al.,* 1986; Voisard *et al.,* 1989; Defago*et al.,* 1990). According to the hypothesis suggested by Davison (1988) cyanide produced by fluorescent pseudomonads inhibits pathogens.

Findings obtained throughout the present work support the results obtained by Attia *et al*. (2016) who found that *P. putida* No.PS23 was the best strain in managing the sugar beet root-infecting *Fusarium oxysporum* f.sp. *betae*. Phenolics were abundantly detected. For instance, vanillic acid, hydroxyethoxy phenol, sinapic acid, hydroquinone, pyrocatechole, gentisic acid … etc. were the most compounds produced by this bacterial strain. Antimicrobial action of phenolic compounds is related to inactivation of cellular enzymes and changing the membrane permeability as stated by Moreno *et al*. (2006). The increase in membrane permeability causes loss of cellular integrity and eventual cell death.

As an alternative to chemical fungicides, safe phenolic antioxidants, benzoic acid and hydroquinone were tested by Abbaas *et al.* (2006) as a foliar spray and led to reduction in the incidence of chocolate spot disease of faba bean caused by *Botrytis fabae.* Benzoic acid inhibited *Rhizoctonia solani* growth *in vitro* and efficiently controlled both pre-emergence damping-off and post-emergence seedling mortality of *Phaseolus vulgaris*.

Phenolic compounds are collectively, comprise several thousand different chemical structures characterized by hydroxylated aromatic ring(s). They are predominantly found in a wide range of commonly plant parts such as fruits, vegetables, cereals and legumes. It is hypothesized that the efficiency of the studied bacterial strains in managing root-infecting pathogens of sugar beet is regarded mainly to the allelopathy of phenolic compounds involved in the secondary metabolites (Rizk*et al.,* 2010).

Phenylpropenes such as ferulic acid is one of the detected chemicals in the growing medium of *P. putida* No. PS23 was previously reported by Provan *et al.* (1994) in significant quantities in the cell walls of many agriculturally important crops such as wheat, maize and sugar beet. It therefore, represents a readily available natural raw material from which the generation of valuable products such as vanillin (Hagedorn and Kaphammer, 1994) may be happened Camphor, related to compound type of

monoterpenoides is one of chemical components secreted by this bacterial strain. Monoterpenes are the main constituents in many of plant essential oils. They are useful as potential alternative pest control agents which are safe and fully degradable pesticides. Their fungicidal effect was reported by Wuryatmo *et al.* (2003). This report supports the findings of the potential of using the bacterial strain (PS23) as biocontrol agent. Phytol, a compound type of diterpenoid, the precursor of vitamin E was found within chemical components of turmeric (*Curcuma longa*), having the potency to suppress fungal growth (Shiyou *et al*., 2011). El-Assiuty *et al*. (2006) isolated and identified a number of eight antifungal compounds related to the type of sesquiterpenes from n-hexane extract of *Cymbopogon proximus*, positively suppressed the growth of *F.verticillioides* and other pathogens of maize. El-Assiuty *et al.* (2007) were, also able to control maize ear rots and minimize mycotoxins accumulation by *C .proximus* extracts.

In conclusion, applying such rhizobacterial strains as biocides to control or decrease root-infecting pathogens is recommended. In particular, *P. Putida* No.PS23 as a superior to the rest strains used in the current study is highly suggested to be used to manage the root diseases, in addition to its potency to promote the growth of sugar beet.

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المضادات الفطرية المنتجة بواسطة سلالات من بكتريا السيدوموناس ذات القدرة على المقاومة الحيوية بأمراض الجذور في الهنجر

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أجريت هذه الدراسة بغرض تحديد مضادات الفطريات المنتجة بواسطة خمسة سلالات من بكتريا السيدموناس الفلوريسنتية المعزولة من ريزوسفير بنجر السكر المقاومة لأمراض الجذور والمتسببة عن الفطر فيوزاريوم اكيسبورم. اتتضح من الدراسة أن هذه السلالات تتفاوت فى إمكانية إنتاج الإنزيمات المحللة لجدر خلايا الفطر المسبب وغيرها من العوامل الأخرى ذات الصلة بالمقاومة. وقد اتضح أن سلالة تابعة لنوع البكتريا السيدموناس بيوتيدا كانت أكثر قدرة على إنتاج هذه العوامل المضادة PS23 بالمقارنة بباقى السلالات تحت الدراسة. هذا وقد ثبت بتحليل المركبات الكيماوية المفرزة بواسطة السلالة المذكورة أنها تنتج العديد من المركبات ذات الصلة بتضاد الفطريات ومقاومة الأمراض النباتية وذلك باستخدام تكنيك ال GC/MS، أغلبها من مركبات فينولية سبق تحديدها فى المقاومة.