SECONDARY COMPOUNDS RELEASED BY RHIZOSPHERIC BACTERIA EXHIBIT FUNGISTATIC EFFECTS AGAINST PHYTOPATHOGENIC FUNGUS.

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ABSTRACT

The aim of conducted research was to devalute the inhibition effect of metabolites secreted by some Rhizospheric bacteria on the growth of two pathogenic fungi: *Rhizoctonia solani* and *Fussarium solani*, 330 bacteria isolates were obtained from the rhizosphere of the wheat, tomato, cowpea and Mt. Atlas mastic tree grown in the Sulaimani. Thirty-five isolates were screened for suppression of phytopathogeneic fungus. Results showed that most of these rhizospheric bacteria were have fungistatic potential in different degrees. The highest inhibition of the linear growth of fungi was noted for *R. solani* and *F. solani,* by *Bacillus cereus* LXJ73 (90.41%), *Bacillus atropheaus* SM-1 (75%) respectively. Six bacterial isolates were selected which have potential effect on the growth of the two studied fungi, five isolates belonging to the *Bacillus* genus and one isolate belonging to the genus *Klebsiella*. these isolates were molecularly confirmed by 16SrRNA, and their activity for inhibiting fungal growth were studied. The extracted metabolities analysied by Gas chromatography-Mass spectrometry (GC-MS), showing that extracts contain different volatile organic compounds as Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-, 2-Pentanone, 4-hydroxy-4-methyl-, Isosteviol methyl ester,2-methyl-, Butanoic acid, 3-methyl-, 3-Pyrrolidin-2-yl-propionic acid, 9-Octadecenamide, (Z)-, Hexadecane, Tridecane, 2,5-Piperazinedione, 3,6-bis(2-methyl propyl)-, 1,2-Benzenedicarboxylic acid, diisooctyl ester, Acetic acid, Dodecane, beta.-D-Glucopyranose, 1,6-anhydro-, Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy)propyl ester and cis-Vaccenic acid. The presence of these componuds indicate that all studied *Bacillus* species and *Klebsiella michiganensis* M1-3-11having antagonistic activity and they can be used for development of biocontrol agents to reducing the phytopathogenic problems and decreasing mycotoxine in contaminated crops.

Keywords: biocontrol, rhizospheric bacteria, fungistatic activity, volatile organic compounds.

مجلة العلوم الزراعية العراقية -2022 :53(5):1174-1183 أنور وآخرون

تأثير التثبيطي للمركبات الثانوية المنتجة من البكتريا الرايزوسفير ضد بعض الفطريات الممرضة

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المستخلص

هدفت الدراسة الحالية تقيم التأثيرالتثبيطي لمركبات الايض المفرزة من بعض بكتريا الرايزوسفير ضد للفطريات الممرضة *Rhizoctonia solani* و *Fussarium solani* عزلت ٣٣٠ عزله بكتيرية من جذور القمح ، الطماطم ، اللوبيا ، و ونبات البطم المزروعة في منطقة السليمانية. تم اختيار 35 عزلة لكبت الفطريات الممرضة للنبات. أوضحت النتائج أن معظم بكتيريا منطقة الجذور لها قابلية تثبيط و قتل الفطريات الممرضة *F. solani* و *R. solani* بدرجات مختلفة كأعلى نسبة ثم (90.41 ٪ *Bacillus cereus* LXJ73 (75 ٪ *Bacillus atropheaus* SM-1 (على توالي .وتم اختيار خمسة عزلات بكتيرية تنتمي للجنس *Bacillus* بناءً على قدرتها على تثبيط النمو في العديد من مسببات الأمراض الفطرية، وعزلة واحدة تنتمي إلى جنس *Klebsiella*. تم تأكيد هذه العزلات جزيئيًا بواسطة 16SrRNA , ومن ثم تحليل المركبات العضوية المنتجة كمستقلبات مضادة للفطريات المستخرجة بواسطة n–Hexan عن طريق تحليل(GC–MS) ، وتم تحديدهما كالتالي: (Pyrrolo [1، 2–a] pyrazine-1 4-(Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-, 2-Pentanone, 4-hydroxy-4-methyl-, Butanoic acid, Isosteviol propyl ester, 9-Lauric acid, 2-(hexadecyloxy)- 3 -(octadecyloxy) Tridecane, 2,5- methyl ester,2-methyl-, Butanoic acid, 3-methyl-, Octadecenamide, (Z)-, Hexadecane, Piperazinedione, 3,6-bis(2-methyl propyl)-, Tridecanoic acid, 12-methyl-, methyl ester, Oleoyl chloride, Tetradecane, Diethyl Phthalate, 1,4:3,6-Dianhydro-.alpha.-d-glucopyranose, 1,2-Benzenedicarboxylic acid, diisooctyl ester, Acetic acid, Dodecane, beta.-D-Glucopyranose, 1,6-anhydro-, Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy)propyl ester and cis- Vaccenic acid , جميع هذه المركبات أظهرت مستويات عالية من كمضادات لفطريات و يمكن أن يستخدم كعوامل للمكافحة الحيوية للحد من تطور الأمراض الممرضة للنبات والتلوث بالسموم الفطرية للمحاصيل.

الكلمات المفتاحية: المكافحة الحيوية، البكتيريا منطقة الجذرية، النشاط الفطري، المركبات العضوية المتطايرة.

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INTRODUCTION

Disease control methods include the elimination of infected plants with subsequent chopping or use of systemic pesticides as part of integrated pest control strategies. However, given the need to consider restrictions on using agrochemicals for plant export and consumption, further research should focus on environmentally friendly alternatives for plant disease management. One of these alternatives is biological control, in which naturally occurring beneficial bacteria with antagonistic activity are used against the causes of disease, Bacterial or fungal strains which isolated from the endosphere or rhizosphere usually having the biocontrol agents concerning with plant diseases (18). Various bioactive compounds are produce by rhizobacteria as a particular interest, and they play an important function in supporting plant growth, productivity, and phtopathogens fungi protection. (4) Reports of rhizobacteria with antagonistic activity against fungal pathogens are widespread in the literature and *Bacillus* spp. and *Pseudomonas* spp. in especial are commonly cited among the most effective biocontrol agents. Multiple mechanisms may be implicated in the antifungal activity of rhizobacteria. Bacteria can outgrow fungi by competing for space or resources, or actively inhibit fungal development by emitting antibiotics in diffusible components (2). It has been shown the bacterial species secrete cyclo lipopeptides with antimicrobial effects, principally from the families of iturine, fengycin, or surfactant. (15) The antifungal effects of iturine and fengycin from *Bacillus subtilis/B. amyloliquefy ancient* was studies against *Fusarium oxysporum* and *F. solani* (6,17). The genus *Bacillus* has been shown to emit compounds, including aliphatic ketones that can impede the growth of several species of *fusarium* (8). The aim of the current study were (1) to isolate and identify the rhizospheric organisms from the rhizosphere of four plants species, wheat, cowpea, tomato, and Mt. Atlas mastic tree; (2) to assess the inhibitory impact of rhizospheric microorganisms from chose plants against the two chosen phaytopathoginic fungus; and (3) to analyze the antifungal characteristics of their organic extracts, and to expand the results for environmentally friendly biocontrol agents.

MATERIALS AND METHODS

Soil sampling: During the planting period between April 2017 and July 2018, soil samples with plant roots were collected from field trials of four different species: tomato (*Solanum lycopersicum* L.), cowpea *(Vigna unguiculata* L.), bread wheat (*Triticum sativum* L.), and Mt. Atlas mastic tree (*Pistactia atlantica*). The samples of the soil taken in triplicate in 20 cm depth and stored at 4 °C.

Bacterial isolation and growth condition

The adhering soil of the roots zone in 3-6 mm thick were taken by sterile forceps from each studied plants, From this, 1gm of the soil was taken into the test tube with 9 ml of sterile distilled water then shaken in a rotary shaker at 150 rpm for 30 minutes before dil ution (11). To determine the colony-forming units (CFU), bacterial cultures were double diluted in a 96-well microplate by transferring 20 µl of bacterial culture into 180 µl Phosphate buffered saline with (dilutions 10^{-1},10^{-2},10^{-3} and 10^{-4}) (16). Then, from each dilution, 20 µl were placed onto the surface of LB agar. Plates were inverted and incubated at 30°C for 24h. For calculate the CFU per milliliter, the average number of bacterial colonies in 20 µl was multiplied by 50, and the dilution factor using the following formula:

CFU ml^{-1} = Average number of colonies for a dilution x 50 x dilution factor

For long-term storage, the bacterial isolates were grown in nutrient broth medium until they reached 10^{-8} and the cells were agitated well then frozen at -80°C in 30% glycerol and 70% nutrient broth.

Microscopic and biochemical identifycation of the bacterial isolates

Microscopic examination was performed by preparing a thin smear, then gram stained with standard gram staining procedures as described by(22) and examined under oil lens(x100 power). Soil rhizosphere bacteria cells appeared in different sizes, shapes, and colors. The pink to red colors are gram-negative and pale purple to dark purple colors are gram-positive. Using VITEK® 2 Systems (bioMérieux, USA) for biochemical identification as the manufacturer's instruct-

tions, the stock culture isolates were sub cultured and the fresh Gram-negative isolate was subcultured on MacConkey agar plates and the Gram-positive isolates recultured on nutrient agar plates for 18 to 24 h at 30°C for activation. A bacterial suspension prepared by mixing a small bacterial colony with 3 ml of a 0.45% sodium chloride solution, and it was adjusted in a range between 0.5-0.63 for Gram-negative and 1.8–2.20 for Gram-positive spore-forming bacilli according to McFarland standard. The prepared test cards and specimen test tubes Placed in the cassette, then within 10 minutes the cassette transfered to the cassette loading station in the Vitek 2 system (23).

Molecular identification of selected isolates and Sequencing

The evaluating 16S rRNA sequences was performed for molecular Identification of bacterial strain by purifying the DNA genome of the bacterial strain through using the DNA purification kit for the bacterial genomic (Geneaid, Taiwan). The16SrRNA amplified by the PCR reaction, using forward BF27 (5'–AGAGTTTGATCCTGG CTCAG-3') and U1492R(5'- GGTTACCTT GTTACGACTT-3'). The PCR condition was performed in 3 stages , 1st stage initial denaturation at 94 °C for 5 min ; 2nd stage in 30 cycles denaturation at 94 °C for 1min, annealing at 52 °C for 1min and extension at 72 °C for 2 min; 3rd stage final extension at 72 °C for 5 min. Then using Easy pure®Quick Gel Extraction kit (Beijing, China)for purification and sequenced (Sangar sequencing, Macrogen South Korea). Then blasted with sequences related species from the NCBI GenBank database.

Antifungal activities of the Isolates

The dual culture assay performed for the antifungal activities of 35 isolates against two phytopathogenic fungi (*R. solani* and *F. solani*). Using potato dextrose agar (PDA) medium, for carring out this a 6 mm plug from the edge of a old culture (5 day) of the pathogenic fungi inoculated at the center of PDA medium and then the fungus growth was steressed by inoculating the isolates in a long streak of 2 cm on the medium surface and 3 cm away from the fungi. Inhibition growth of the fungal was measured after incubation for 7 days at temperature 28-30°C and compared with that of the control (i.e., without the bacterial isolate). Isolates that showed significant antifungal activity against the phytopathogens in this tests were repeated in three replicates, thus, the inhibition rate was measured in percentage according to the formula of (9).

Isolate cultivation and crude extraction of bioactive compounds

Based on their antagonistic activities the most active isolates against the two selected fungi were cultivated for crude extraction of their active compounds. The selected isolates were cultured on a Luria Bertani plate (LB). The cultured plates were incubated at 30°C for 24hr (with 3 replicate). Each actively growing pure culture of the isolates was used to inoculate 100 ml of Luria Bertani broth (1). The extraction were performed by using the method (1) with some modifications, 10% of the cultured in LB broth were taken in to be used as a seed culture for fermentation medium in three separate 150 ml of (yeast extract, glucose, NaCl, oatmeal, $CaCO_3$, at pH 7.0). Then the cultures were incubated at 30 °C for 10 days inshaking incubators of 180 rpm. Then the medium was centrifuged for 30 min at 6000 × g to separate the bacterial cells. The supernatant was mixed with anequal volumes of n-hexane as an organic solvent, then shacked for 30 min. and separated in a separating funnel. The solvent was removed by a rotary evaporator.

Analysing of crude cell extract by GC-MS

Using GC-MS- QP2010 Ultra, (Shimadzu Co., Japan) for Identification and analysing of the bacterial metabolites. the helium flowed at 1 ml/min through an instrument supported with an Rtx-5ms column (30 m × 0.25 mm ID, 0.25 μm film thickness). The diluted sample was heated at 60°C for 30 min and 1 ml of the headspace was injected using a gas syringe (21). the temperature had been increased at 6°C per min, then the column was saved at 40°C for 2 min, then at 250°C for 13 min. The injection port was at 200°C and interface at 250°C. The chromatogram and mass spectra were recorded and analyzed. The m/z peaks representing mass to charge ratio charac teristic of the antimicrobial fractions were compared with those in the mass spectrum of NIST (National Institute for Standards and

Technology) library of the correspon ding organic compound.

Statistical analyses

All data were statisticaly analysied by using one-way ANOVA with 0.05 probability. Ap - plying the Duncan test for comparing the means of the values among the treatments with one another.

RESULTS AND DISCUSSION

Characterization of rhizosphere bacteria

Plant growth promoting rhizobacteria (PGPR) were isolated from the rhizosphere of tomato (*Solanum lycopersicum* L.), cowpea *(Vigna unguiculata* L.), bread wheat (*Triticum sativum* L.), and Mt. Atlas mastic tree (*Pistactia atlantica*). Pure colonies were identified by morphological characteristics and biochemical methods. One hundred fourteen, 89, and 127 isolates are Gram-positive, Gram-negative, and bacilli spore, respectively, as shown in Figure (1)

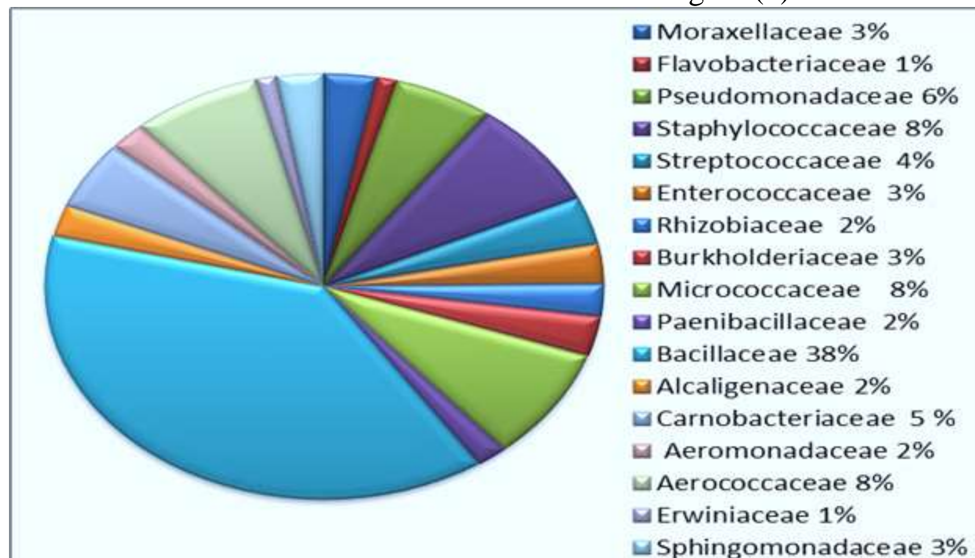

Figure 1. Frequency of bacterial isolates from the rhizospher of selected plants represented by percent for each family

Bacterial isolates with antifungal activity were confermed thier identification molecularly by sequencing their 16S rRNA gene for more confirmation. The partial sequences were ranged between 1430-1545bp,with asimilarity of 98% - 100% of the blasted sequence.

Antagonistic activity against the studied pathogenic fungi

The inhibition of pathogen mycelial growth and suppression of spore germination concedred as a results of antigonistic activities of the rhizosphere bacteria against phytopathogens fungies. These 330 bacterial isolates are distinguished into 17 groups according to its response to the Gram-stain, and microscopic characteristics like size and cell shape of the bacterial cells were also done 35 isolates from the total bacterial isolates were selected randomly that obtained from four plant species, they were cheaked for thier antagonistic activity by growing each isolates with *R. solani*, and *F. solani* pathogens in one plates . The results of inhibition percentages of fungal growth by the 35 bacterial isolates are appeared in (Table-1). A significant difference in the growth inhibition was observed among the isolates. The radial growth of mycelial of the *R. solani* and *F. solani* pathogens were inhibited by all studied isolates, with inhibition percentages ranges from 20.83 to 90.42% and 41.25 to 75.00% against *R. solani*, and *F. solani,* respectively. Bacterial isolates with the local number 9, 20, and 6 from 35 isolates showed the value of growth suppression between 10-50, 50-80, and 80-100%, respectively, in *R. solani*. Whereas, 14, 13 and 8 isolates of 35 bacterial isolates exhibited a range of inhibition of growth from 30 to 50, 50 to 60, and 60 to 80% against *F. solani.* The highest inhibition of growth (90.42%) against *R. solani* was showed by *Bacillus cereus* LXJ73 followed by *Bacillus licheniforms B27* (86.66%) and *Bacillus licheniformis 1* (86.66%), while the maximum value of the growth inhibition (75%) in *F. solani* was displayed by *Bacillus atropheaus* SM-1 followed by *Bacillus licheniforms* KUBOT- AB1(66.87%) and *Klebcilla michiganensis M1-3-11*(65.41%). Chemical

pesticides have helped farmers reduce crop losses caused by microbial phytopathogens, but they have also been linked to deforestation, the evolution of resistancy of the pathogens, and threats to validity of the human. Several studies have suggested the use of useful rhizospher bacteria as a biological control agents to solve the problems caused by phytopathogenic fungi and to reduce the harmful belongings of pesticide or a chemical fertilizer (18,6,2). (11) also showed growth inhibition of *R. solani* by *B. licheniforms* and *B. pumillus.* recently discovered that Bacillus VOCs inhibited many fungal growths in different degrees. In another investigation by(12) found that the severity of the pathogens was significantly reduced. Furthermore, our findings are came in agreement with the reporting of (14), who found *B. Pumilus* SMH101 has a strong antifungal spectrum against *F. solani, R. solani,* and *C. albicans*.

Extract analysis by GC-MS

The GC–MS analysis indicated that out of the six strains found, *K. michiganensis* M1-3-11, *B. licheniforms* KUBOTAB1, *B. licheniforms* B27, *B. cereus* LXJ73, *B. sonorensis* KW50P, and *B. atroupheus* SM1, *B. atroupheus* SM1 had the most metabolites with 27, *B. licheniforms* B27 had the fewest metabolites, with just five, while isolates *K. michiganensis* M1-3-11, *B. licheniforms* KUBOTAB1, *B. cereus* LXJ73, and *B. sonorensis* KW50P had 20, 14, 24, and 26 metabolites, respectively. some organic compounds were repeteadlly detected by the four isolates, *Klebsiella michiganensis* M1-3-11, *B. licheniforms* B27, *B. sonorensis* KW50P and *B. atroupheus* SM1 is pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-. Others compound repeted to *B. licheniforms* KUBOTAB1, *B. cereus* LXJ73 undecane. Those common to *K. michiganensis* M1-3-11, *B. licheniforms* KUBOTAB1is Tridecane. The predominated components in different isolates extracts were: 2-Pentanone, 4-hydr oxy-4-methyl- and Pyrrolo[1,2 a]pyra zine-1,4-dione, hexahydro- in *K. michiganensis* M1-3-11, undecane, Bis-(3,5,5-trimethyl hexyl) phthalate, Butanoic acid, 3-methyl- andPyrrolo[1,2-a]pyrazine-1,4-dione, hexa hydro- in *B. licheniforms* KUBOTAB1, 3-Octanone, 8-(6-tricosyl-2-methyl-1,3-dioxan -4-yl)-4-methyl-,[4R-[4.alpha. (R*),6.beta.]]- and Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy)propylester in *B. licheniforms* B27, pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro- and 9-Octadecenamide, (Z)- in *B. cereus* LXJ73, Butanoic acid, 3-methyl- and pyrrolo[1,2-a]pyrazine-1,4-dione, hexa hydro- in *B. sonorensis* KW50P, and Butanoic acid, 3-methyl-, Butanoic acid, 2-methyl- and pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- in *B. atroupheus* SM1. A variety of compounds have several biological pro perties including cytotoxicity, antioxidant, anti-inflammatory, antimicro bial, and anti viral activities were observed (25). Isolated 3-Pyrrolidin-2-yl-propionic acid as a bioactive organic compound from *Klebsiella michiganensis* M1-3-11**,** *B. sonorensis* KW50P, and *B. atroupheus* SM1 showed its antifungal activity against aflatoxin producing Aspergillus by (5). *B. cereus* LXJ73 formed hexadecane, which had antimicrobial, antifungal, and antioxi dant properties (26). *B. licheniforms* B27 and *B. cereus* LXJ73 produced the saturated fatty acid lauric acid, which repoted that suppressing the growth of *R. solani* (24). Dodecane, which was found in *B. cereus* LXJ73, had antifungal properties (19).Penta decane was detected in *B. cereus* LXJ73 displayed for *F. oxysporum* growth according to (27). In a study by (12) showed significant antimicrobial activity of *B. licheniforms.*

Table 1. The inhibition zone (%) caused by PGPR against some phytopathogenes fungus

Name	Inhibition of Growth %	
	Rhizoctonia solani	*Fusarium solani*
Bacillus cereus LXJ73	90.417 a	62.500 abcd
Bacillus licheniforms B27	86.667 ab	61.250 abcde
Bacillus licheniformis1	86.667 ab	54.167 bcdef
Bacillus atropheaus SM-1	83.333 ab	75.000 a
Bacillus sonorensis Kw50p	81.250 ab	57.500 abcdef
Bacillus zhangzhouensis QI_110	80.000 ab	65.000 abc
Klebcilla michiganensis M1-3-11	79.167 ab	65.417 abc
Bacillus safensis JLs5	77.083 ab	47.917 bcdefgh
Bacillus pumillus 3-19	75.000 abc	56.250 abcdf
Bacillus licheniforms KUBOTAB1	75.000 abc	66.875 ab
Leuconostoc mesenteroides /	72.917 abcd	57.083 abcdef
Kocuria kristinae	72.083 abcd	50.000 bcdefgh
Bacillus halotolerans SY1836	70.833 abcde	57.917 abcdef
Bacillus cereus	68.750 abcdef	63.333 abcd
Sphingomonas paucimobilis	68.750 abcdef	50.417 bcdefg
Bacillus fortis 1	66.667 abcdefg	48.750 bcdefgh
Bacillus subtillus MN524117.1	66.667 abcdefg	59.583 abcdef
Enterobacter cloacae	66.667 abcdefg	54.167 bcdef
Paenibacillus glucanolyticus	65.417 abcdefg	40.417 fgh
Pseudomonas putida	64.583 abcdefg	46.667 cdefgh
Klebsiela pneumonia	60.417 abcdefgh	53.333 bcdef
Bacillus coagulans	60.417 abcdefgh	49.167 bcdefg
Bacillus megaterium	60.417 abcdefgh	60.417abcde
Pseudomonas fluorescens	60.417 abcdefgh	58.333abcdef
Rhizobium radiobacter	56.250 abcdefgh	33.750 gh
Burkholderia giadioli	52.083 abcdefgh	47.917 bcdefgh
Coronobacter sakazakii /	47.917 bcdefgh	45.417 defgh
Geobacillus toebii	36.250 cdefgh	44.167 defgh
Brevibacillus laterosporus	34.167 defgh	31.250 h
Moganella morganii	30.417 efgh	47.917 bcdefgh
Alloiococcus otitis	30.000 fgh	34.167 gh
Pantoea spp	29.167 fgh	57.292 abcdef
Bacillus gelatini	27.917 gh	47.917 bcdefgh
Virgibacillus pantothenticus	27.083 gh	56.667 abcdef
Streptococcus suis L	20.833 h	42.500 efgh
Pr > F (Strains)	< 0.0001	< 0.0001
Significant	Yes	Yes

Table 2. Characterization of VOCs emitted by *Klebsiella michiganensis* M1-3-11, analyzed by –GC-MS

No. peak	Compound	RT(min)	RA(%)
1	2-Pentanone, 4-hydroxy-4-methyl-	3.187	37.6
2	Oxime-, methoxy-phenyl-_	3.582	0.33
3	2-[4-Chloro-trans-styryl]-6-chloro-5-[4-chlorophenyl]-4-[3,5 bis[pyrrolidinomethyl]-4-hydroxyanilino]	4.148	0.41
4	Rhodium, di-.mu.-chlorobis[(1,2,5,6-.eta.)-1,5-cyclooctadiene]di-	4.262	0.42
5	N-Benzyl-2-{2-[(benzyl-phenyl-carbamoyl)-methoxy]-1,2-diphenyl-ethoxy}-N-phenyl-acetamide	4.61	0.44
6	Phenylethyl Alcohol	5.249	0.83
7	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	6.026	1.01
8	2,4,4-Trimethylbut-2-enolide	6.557	0.78
9	Tridecane	7.209	0.56
10	.beta.-D-Glucopyranose, 1,6-anhydro-	8.055	1.32
11	Tetradecane	8.497	3.83
12	1-Oxaspiro[4,5]decane 4-carbonitrile, 2-oxo-	9.351	0.42
13	3-Pyrrolidin-2-yl-propionic acid	9.56	1.79
14	3-Pyrrolidin-2-yl-propionic acid	9.793	1.21
15	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	10.011	33.19
16	2-(E)-Heptenoic acid, (4S)-4-[((R)-alanyl)amino]-6-methyl-	12.175	3.14
17	Piperazine, 1,4-bis(1-oxooctadecyl)-	12.275	0.95
18	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	14.786	7.3
19	Ketone, vinyl-pyrrolidinyl-	15.076	2.37
20	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'.alpha.,10.alpha.)-	17.645	2.09

Table 3. Characterization of VOCs emitted by *B. licheniforms* KUBOTAB1, analyzed by –GC-MS

No. peak	Compound	RT(min)	RA(%)
1	Butanoic acid, 3-methyl-	3.052	7.73
2	1-Pentacontanol	3.293	1.45
3	1-Pentanol, 4-methyl-2-propyl-	3.593	2.95
4	1-Decanol, 2-ethyl-	3.836	4.72
5	.beta.-Thionaphthol maltoside heptaacetate	3.925	1.7
6	Nonane, 2-methyl-	4.175	10.17
7	Heptadecane, 2,6-dimethyl-	4.72	2.25
8	Undecane	5.01	11.09
9	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	6.025	3.66
10	Naphthalene, decahydro-2,6-dimethyl-	6.071	4.09
11	Naphthalene, decahydro-1,5-dimethyl-	6.112	4.67
12	Tridecane	7.209	6.91
13	Bis-(3,5,5-trimethylhexyl) phthalate	8.493	8.31
14	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	10.013	30.29

Table 4. Characterization of VOCs emitted by *B. licheniforms* B27, analyzed by –GC-MS

No. peak	Compound	RT(min)	RA(%)
1	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	10.01	3.1
2	3-Octanone, 8-(6-tricosyl-2-methyl-1,3-dioxan-4-yl)-4-methyl-, [4R-[4.alpha.(R*),6.beta.]]-	25.094	8.15
3	Cholestano[3,2-c]isoquinolin-1'(2'H)-one, 3',4'-dihydro-6',7'-dimethoxy-	25.85	2.22
4	3-Octanone, 8-(6-tricosyl-2-methyl-1,3-dioxan-4-yl)-4-methyl-, [4R-[4.alpha.(R*),6.beta.]]-	26.981	13.56
5	Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy)propyl ester	27.935	72.96

Table 5. Characterization of VOCs emitted by *B. cereus* LXJ73, analyzed by –GC-MS

No. peak	Compound	RT(min)	RA(%)
1	Butanoic acid, 2-methyl-	3.159	6.3
2	Octane, 4-ethyl-	3.595	1.29
3	3-Carbethoxy-6-n-butyl-7-octadecylmercapto-4-quinolone	4.363	3.64
4	Undecane	5.01	3.96
5	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-	5.442	9.63
6	Undecane	5.791	6.93
7	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	6.019	2.18
8	Bicyclo[2.2.2]octane, 1,2,3,6-tetramethyl-	6.075	2.15
9	Naphthalene, decahydro-1,5-dimethyl-	6.111	2.37
10	Pentadecane	7.208	2.59
11	.beta.-D-Glucopyranose, 1,6-anhydro-	8.049	2.81
12	Hexadecane	8.497	2.83
13	1,3,5-Cycloheptatriene, 2,5-dihexyl-7,7-dimethyl-	8.769	1.93
14	1,3,5-Cycloheptatriene, 2,4-dihexyl-7,7-dimethyl-	8.93	2.16
15	Dodecane, 1,1'-thiobis-	9.827	3.14
16	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	10.008	21
17	Phthalic acid, butyl undecyl ester	11.785	4.01
18	N-Isopentylidene-2-butylamine	15.087	2.72
19	9-Octadecenamide, (Z)-	17.294	11.24
20	Hexanedioic acid, mono(2-ethylhexyl)ester	17.657	1.72
21	Isosteviol methyl ester	18.679	0.32
22	Methyl steviol	18.877	0.93
23	Lauric acid, 2-methylbutyl ester	19.037	1.14
24	1,2-Benzenedicarboxylic acid, diisooctyl ester	19.703	3.01

Table 6. Characterization of VOCs emitted by *B. sonorensis* KW50P, analyzed by –GC-MS:

No. Peak	Compound	RT(min)	RA(%)
1	Butanoic acid, 3-methyl-	3.197	28.36
2	Butanoic acid, 2-methyl-	3.248	8.03
3	Evonine	3.483	0.51
4	Oxime-, methoxy-phenyl-_	3.525	0.44
5	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	6.023	1.8
6	Naphthalene, decahydro-1,5-dimethyl-	6.17	1.03
7	Bicyclo[2.2.2]oct-5-ene-2-carbonitrile, 2-chloro-	7.568	0.73
8	Phenol, 2,4-bis(1,1-dimethylethyl)-	7.947	0.59
9	.beta.-D-Glucopyranose, 1,6-anhydro-	8.042	3.67
10	Benzaldehyde, 4-(dimethylamino)-	8.292	1.62
11	d-Arabino-hexonic acid, 2-deoxy-3,4,5-tris-O-(trimethylsilyl)-, trimethylsilyl ester, bis(trimethylsilyl)	8.533	1.1
12	o-Acetyl-N,o'-carbonyl-tetrahydro-solasodine	9.348	0.67
13	Dotriacontyl trifluoroacetate	9.566	1.97
14	3-Pyrrolidin-2-yl-propionic acid	9.796	2.24
15	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	10.005	24.68
16	Pentadecanoic acid	10.378	1.2
17	6-Oxabicyclo[3.1.0]hexan-3-one, 2,2,4,4-tetramethyl-	10.447	1.36
18	n-Hexadecanoic acid	11.862	2.36
19	Nitro-L-arginine	12.192	2.82
20	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	14.792	2.21
21	2-Decene, 3-methyl-, (Z)-	15.075	5.61
22	9-Octadecenamide, (Z)-	17.294	1.81
23	5-Ethyl-1-nonene	17.656	1.11
24	Isosteviol methyl ester	18.683	1.59
25	Methyl steviol	18.875	0.62
26	1,2-Benzenedicarboxylic acid, diisooctyl ester	19.705	1.86

Table 7. Characterization of VOCs emitted by *B. atroupheus* SM1, analyzed by –GC-MS

No. peak	Compound	RT(min)	RA(%)
1	Butanoic acid, 3-methyl-	3.225	18.01
2	Butanoic acid, 2-methyl-	3.309	10.76
3	3-Pyridinemethanamine	7.566	0.17
4	3,6-Dimethylpiperazine-2,5-dione	8.237	1.41
5	Diethyl Phthalate	8.469	0.84
6	[1,3]Diazepan-2,4-dione	8.558	1.65
7	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	8.897	2.96
8	5-Methoxypyrrolidin-2-one	9.358	4.1
9	3-Pyrrolidin-2-yl-propionic acid	9.569	3.47
10	3-Pyrrolidin-2-yl-propionic acid	9.808	1.42
11	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	10.08	29.21
12	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	11.645	1.3
13	N(1)-(3-Methyl-1,2,4-oxadiazol-5-yl)-1-pyrrolidine carboxamidine	11.791	2.35
14	Nitro-L-arginine	12.223	2.96
15	Hexahydro-2(1H)-azocinone	13.004	1.98
16	9-Octadecenamide, (Z)-	13.398	0.91
17	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	14.062	1.22
18	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	14.821	1.89
19	Hexanal, 3-(hydroxymethyl)-4-methyl-	15.103	2.06
20	1,9-Dioxacyclohexadeca-4,13-diene-2-10-dione, 7,8,15,16-tetramethyl-	15.667	0.62
21	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'	17.024	0.45
22	7(8H)-Pteridinone, 6-methyl-	17.142	3.4
23	9-Octadecenamide, (Z)-	17.1301	0.88
24	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	17.66	0.58
25	7(8H)-Pteridinone, 6-methyl-	19.676	2.05
26	Lupeol	26.494	0.65
27	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	28.651	2.71

Conclusion

The determination of different biological active compounds Qualitativly from crude extractes of 6 selected rhizospheric bacteria by using GC-MS indicated of different chemical compounds with high molecular weight and low molecular weight in varying amounts revealed in each of the bacterial extracts. 6 identical VOCs are detected in both *Bacillus* and *Klebsiella*. These active compounds are important biologically as antibiotic, antifungal compounds by inducing systematic resistance of plants important. The inhibition of pathogen mycelial growth and suppression of spore

germination concedred as a rsults of anta - gonistic activities of the rhizosphere bacteria against phytopathogens fungies. these results are a best example of the biocontrol mechanisms by rhizospherer bacteria.

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