Induction Of Resistance And Biocontrol Of Rhizoctonia In Cotton Damping-off Disease By Rhizosphere Bacteria And Actinomycetes

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Citation

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Abstract

Addition of fishmeal to the soil infested with the pathogen led to a remarkable reduction in the percentage of disease compared to the soil non-amended with fishmeal. 43 isolates of actinomycetes and 8 isolates of bacteria were isolated from the rhizosphere associated soil of cotton plant. Four actinomycete isolates were found to have a potent antagonistic activities against Rhizoctonia solani
and were identified as Streptomyces erumpens, S. purpureus, S. aurantiacus and S. microflavus.

Four bacterial isolates were found to have strongest antagonistic activities and were identified as Arthrobacter ramosus, Aureobacterium terregens, Curtobacterium pusillum and Pseudomonas

putida. The use of a mixture of actinomycete and bacterial species inhibit the pathogen , increase the induction of resistance in cotton plant and increase the growth measurements of cotton plant than each alone. S. purpureus and A. ramosus

have the abilities of solubilizing phosphate and have the abilities for the production of both siderophores and indolacetic acid (IAA).The four bacterial species, each produce 5 antifungal compounds. On the other hand, one of the four Streptomyces species produce 5 antifungal compounds ,while the other three species each produce 4.

INTRODUCTION

The present investigation was carried out to study the biological control of damping-off disease in cotton, caused by Rhizoctonia solani, by rhizosphere actinomycetes and bacteria through some mechanisms which are induction of resistance, antibiosis and soil amendment.

REVIEW OF LITERATURE

Ten Streptomyces species were selected from 386 isolates of rhizosphere fungi and actinomycetes because of their antagonism to Fusarium oxysorum, Phymatotrichum omnivorum, Rhizoctonia solani, ad Verticillium albo-atrum. Six Streptomyces species produced candicidin, a heptaene antibiotic, where as four species produced unknown heptaenes or cyclohexamide-candicidin mixtures [49].

Fluorescent pseudomonads produce a yellow-green pigment, a siderophore, designated as “pseudobactin”. The role of pseudobactin in promoting growth of potato was demonstrated when 10 µg of pseudobactin promote growth to the same extent as when fluorescent pseudomonads were applied to potato seed pieces [32].

Bacterial biocontrol agents enhance plant growth and reduce disease by utilizing a number of different mechanism; these include the production of antibiotics and toxins that reduce pathogen growth and infection potential, competition for infection sites and induction of resistance mechanisms in the plant [3].

Subpopulation of rhizosphere bacteria from numerous agricultural plants produce antifungal compounds in vitro. Studies have been done to characterize antagonistic bacterial

populations that reside in the rhizosphere of maize and cotton. Frequently isolated genera that produced antifungal compounds included Pseudomonas, Bacillus, Erwinia, Enterobacter and Serratia [33].

There is an evidence that organic amendments applied in field soils can suppress soil borne diseases. Disease suppression includes the reduction of both disease incidence and severity [37].

Isolates of Streptomyces ambofaciens were able to control Pythium damping-off in tomato plants and Fusarium wilt in cotton plants in an artificially infested soil [15].

Non-pathogenic rhizosphere-colonizing Bacillus and Pseudomonas species induced systemic resistance in cucumber (Cucumis sativus L.) against Pythium aphanidermatum (Edson) Fitzp [14].

An antifungal bacterial strain, isolated from a greenhouse soil sample, inhibits growth of microflora nearby. It was selected for further studies of bacterial antifungal properties. This isolate was identified as Pseudomonas sp. Which exhibits antifungal activity against the plant pathogens, Pythium ultimum, Rhizoctonia solani, Phytophthora capsici, Botrytis cinerea and Fusarium oxysporum [29].

Some actinomycetes species were selected from the rhizosphere of healthy tomato and kidney bean plants capable of inhibiting the growth of some pathogenic fungi. They found that all the tested actinomycete spp. had an inhibitory effect on Alternaria solani greater than on Fusarium solani. Steptomyces graminofaciens was the best one concerning the inhibitory effect on both fungal pathogens [2].

Bacteria were isolated from a cultivated soil and screened for antagonistic activity against Fusarium grameniarum, a predominant agent of ear rot and head blight in cereal crops. Based on its in vitro effectiveness, isolate D1/2 was selected for characterization and identified as Bacillus subtilis by phenotypic tests and comparative analysis of its 16S ribosomal RNA gene (rDNA) sequence [54]

MATERIALA AND METHODS

SOIL SAMPLES

Clay soil was collected from a field cultivated with cotton plant from Shebeen el-Kanater, Kliubiia goveronrate, Egypt.

The clay soil was used for cultivation of cotton plant in the greenhouse and was divided into two categories; the first one was amended with fishmeal and the second one was left non-amended. The rhizosphere soil samples were collected from both amended and non-amended soil.

ISOLATION OF THE PATHOGEN ()

Cotton seedlings one month-old exhibiting damping-off symptoms were collected from soil infested with R. solani. Isolation was made according to Carling et al., [12].

IDENTIFICATION OF THE PATHOGEN ()

Colony morphology of R. solani was assessed on potato dextrose agar (PDA), and Sabourod agar at 28±2ºC in the dark for 2 days. Identification of the pathogen isolate was based on morphological characteristics of the culture growing on the two media [39>,41].

INOCULUM PRODUCTION OF THE PATHOGEN

Sterilized millet (Panicum miliceum L.) was inoculated with agar plugs from the actively growing margin of Rhizoctonia colony and incubate at 28± 2ºC for 6 days in the dark. Sterilized uninoculated millet seeds were used as control [52].

SOIL INFESTATION:

Four sets of pots were used. The first set contains the pathogen and soil amended with fishmeal, the second set contains the pathogen and non-amended soil, the third set contains non-amended soil without pathogen (control), while the last set contains amended soil without the pathogen. 10 seeds were sown in each pot at 3 cm depth, reduced to 5 seedlings per pot after complete emergence. Each treatment was replicated 6 times with 5plants/replicate. The pots were watered every other day [34].

SOIL PREPARATION

Two sets of pots were prepared, each of 10 pots. The pots of the first set contain amended soil and the pots of the second set contain non-amended soil. 10 seeds of cotton were sown at 3 cm depth in each pot. The pots were watered every other day and after 3-4 weeks, the root systems were cut and washed [27].

ISOLATION OF RHIZOSPHERE ACTINOMYCETES AND BACTERIA

Actinomycetes and bacteria of cotton rhizosphere soils (amended and non-amended) were isolated using the soil dilution plate method of [15>,27>,51] on (i) starch –nitrate agar (sna), (ii) starch-casein agar (sca) and yeast extract-malt

extract agar (yema) for actinomycetes and on (i) king;s medium b, (ii) nutrient agar (na) and (iii) 1.5 m32 for bacteria.

SCREENING OF ANTIFUNGAL ACTIVITY OF ACTINOMYCETE AND BACTERIAL ISOLATES

Actinomycete and bacterial isolates were examined for their ability to produce inhibitory compounds active against R. solani using the dual culture agar method [24].

IDENTIFICATION OF RHIZOSPHERE ACTINOMYCETES AND BACTERIA

A- IDENTIFICATION OF ACTINOMYCETES

From the actinomycete isolates recovered from the rhizosphere soils (amended and non-amended) four were found to be strongly antagonistic to R. solani in vitro. These isolates were identified and grouped to genus level on the basis of their standard morphological criteria and according to the absence or presence of aerial mycelium, distribution (on aerial and substrate mycelia) and form of any spores present and stability or fragmentation of the substrate mycelium [16]. these four genera were then identified to the species level.

MORPHOLOGICAL CHARACTERISTICS

The actinomycete isolates were examined morphologically as described by [50].

SPORE SURFACE ORNAMENTATION:

Spore surface ornamentation was determined by examining spores by transmission electron microscope [46]. this was carried out at the central laboratory of the faculty of science, ain shams university.

CULTURAL CHARACTERISTICS:

The type and intensity of growth, color of aerial and substrate mycelia were determined for the actinomycete isolates using the 7 color Wheels of Tresner and Dackus [47]. these were determined for cultures grown on sna, glycerol-asparagine agar (gaa), oatmeal agar, yema, na, glucose asparagine agar and hussein's fishmeal extract agar (hfmea) [25] and incubated at 28ºc in the dark.

IDENTIFICATION OF RHIZOSPHERE BACTERIA

The selected bacterial isolates were identified according to “Bergey's manual of systematic bacteriology” [6]. identification techniques were carried out according to the recommended methods in “medical laboratory manual for tropical countries” [13], “manual for identification of medical bacteria” [17], and “microbiology laboratory manual” [11].

Morphological characteristic of individual colonies were recorded, and then Gram staining was applied to the bacterial isolates. Motility was observed microscopically by hanging drop technique. Several physiological and biochemical tests were carried out according to the identification schemes in “Bergey's manual of systematic bacteriology” [6].

INOCULUM PRODUCTION OF SELECTED RHIZOSPHERE ACTINOMYCETES AND BACTERIA:

The inoculum of each actinomycete and bacterium was prepared by placing 50 g of moist wheat bran and vermiculite into 500 ml conical flasks autoclaved at 121ºC for 30 min. on three successive days as described by Roiger and Jeffers [42]. the mixture of wheat bran or vermiculite and water was inoculated aseptically with spore suspension (25 ml) of each actinomycete in 10% glycerol and a suspension of 25 ml of bacterial cells and incubated at 28±2ºc in the dark for 2 weeks in case of actinomycetes and for 2 days in case of bacteria. the flasks were shaken to ensure uniformity.

SOIL INFESTATION

Wheat bran with each actinomycetes and vermiculite with each bacterium (0.5% weight of colonized wheat bran or vermiculite based inoculum/weight of air dry steam-pasteurized soil) was thoroughly dispersed through the steamed soil contained in two sets of pots in which each pot was filled with 3 kgs clay soil. One set of pots contained the antagonist inoculum and soil amended with fishmeal and the other contained the antagonist inoculum and non-amended soil. In total, there are 11 pathogen-actinomycete and pathogen-bacteria, these were as follows:

R. solani + Streptomyces aurantiacus. R. solani + S. erumpens. R. solani + S. microflavus R. solani + S. purpureus. R. solani + Mix. of four actinomycetes. R. solani + Arthrobacter ramosus. R. solani + Aurobacterium terregens. R. solani + Curtobacterium pusillum . solani + Pseudomonas putida. 10- R. solani + Mix. of four bacteria. 11- Pathogen alone.

Effect of the selected rhizosphere actinomycetes and bacteria on cotton plant growth:

COTTON GROWTH ASSESSMENT

Cotton seedlings were harvested 4 weeks after seedling emergence. The whole plants were carefully removed as washed to remove any soil particles from the shoot and root systems. Growth criteria measures were: length of root, height of shoot, fresh weight of root, fresh weight of shoot, dry weight of root and dry weight of shoot.

STATISTICAL ANALYSIS

A randomized complete block design was used and analysis of variance was carried out using Superanova (Abacus Concepts, Inc., Berkeley, CA, USA) to evaluate the effect of the antagonists on the development and growth of cotton plant in the greenhouse trials.

EFFECT OF THE SELECTED RHIZOSPHERE ACTINOMYCETES AND BACTERIA ON PHOSPHATE SOLUBILIZATION

The medium used to screen phosphate solubilization consists of soil extract 75 ml with 1% glucose and 2% agar, dispensed in 300 ml amounts in 500 ml Erlenmayer flasks. After sterilization, the medium was cooled to 45ºC and 15 ml of sterile 10% K2HPO4 and 30 ml sterile 10% CaCl2 were added. Two ml of sterile actidione solution (40 µg/ml) were also added to each flask and the reaction adjusted aseptically with sterile N/L NaOH at a pH 7.0. The plates were inoculated with the rhizosphere actinomycetes and bacteria and incubated at 28ºC for 5 days. The organisms forming clarification halos were considered phosphate solubilizers [$_{28}$].

PRODUCTION OF SIDEROPHORES

The medium used to test for production of siderphores consisted of 8-hydroxyquinoline (50 mg/L) was added to tryptic Soy Agar (TSA) (10%). Organisms growing on this medium were considered positive for siderophores production [$_{4}$]

PRODUCTION OF IAA AND/OR IAA ANALOGS

A modified method [$_{8}$] was used to screen indolacetic acid (iaa and/or iaa analog producers. tsa (10%) amended with 5 mm 1-tryptophane was overlaid with an 82-mm diameter nitrocelluolse membrane disk. agar plates were inoculated with tested isolates and incubated at 28ºc for 3 days. the membranes were overlaid on a whatman no. 2 filter paper saturated with salkowski reagent (1.0 ml of 0.5 m fecl3 + 50 ml 0f 35% hclo4) [$_{21}$].

EXTRACTION OF THE ANTIFUNGAL SUBSTANCES

The selected actinomycete and bacterial species were cultivated on HFME broth, culture broth was then cultivated and extracted with a mixture of chloroform-ethyle acetate (1:1 v/v). Complete extraction was achieved when the metabolism solution was extracted with 30% of its own volume of chloroform-ethylacetate mixture. The organic layer containing the antifungal substances was collected and evaporated at 34ºC under vacuum till dryness. The residue was dissolved in the least amount of chloroform (3 ml) [$_{40}$].

DETECTION OF HE ANTIFUNGAL SUBSTANCES

The active components in the culture filtrates of the selected actinomycete and bacterial species were studied by means of descending paper chromatography, the solvent system was 1-butanol, pyridine, and water (6:4:3 v/v).

After development, the paper strips (Whatman No. 1) were air dried and placed in front of a strip of R. solani growing for three days on PDA. After incubation, the antifungal components were detected according to their Rf values [$_{40}$].

CHEMICAL STRUCTURE OF THE PURIFIED ACTIVE COMPONENTS OF THE SELECTED ACTINOMYCETE AND BACTERIAL SPECIES

This experiment was carried out according to [$_{18}$], using mass spectroscopy. varian gas chromatography coupled with as mass selective detector. finningan mat ssq 700 and equipped with chem-station soft ware and nist spectral data was used with db-5 fused silica capillary column (30 x 0.25 um i.d., 0.25 um film thickness). the chromatographic conditions were as follows: column temperature 60°c (30 min), raised from 60 t0 260°c (5°c/min.) and maintained at 260 for 10 min., interface, 260°c; injector temperature 250°c, ionization energy 70 ev; mass range 50-750; volume injected 1 ul. this experiment was performed at the mass spectroscopy unit, central scientific services laboratory at the national research center, cairo, egypt.

RESULTS

SCREENING THE ACTINOMYCETE AND BACTERIAL ISOLATES FOR ANTAGONISTIC ACTIVITIES AGAINST .

Actinomycete isolates No. 23, 24, 31 & 41 were found to have the most potent and inhibitory effect on R. solani than the other isolates. On the other hand, bacterial isolates No. 1,

3, 7 and fluorescent isolate exhibit higher percentage of inhibition than the other isolates (Tables 1 & 2).

IDENTIFICATION OF ACTINOMYCETES

The four strongest antagonistic isolates were identified to the genus and species levels as (23) Streptomyces erumpens, (24) S. purpureus, 3(31) S. aurantiacus and (41) S. microflavus. The four strongest bacterial isolates were identified to the genus and species levels as ((1) Arthrobacter ramosus, (3) Aureobacterium teregenes, (7) Curtobacterium pusillum and the fluroescent isolate as Pseudomonas putida.

EFFECT OF ACTINOMYCETES AND BACTERIAL SPECIES ON COTTON SEED GERMINATION USING AMENDED AND NON-AMENDED SOILS

It is clear from table (3) that infestation of soil with the pathogen led to a reduction in the percentage of seed germination compared to the non-infested soil (control), indicating that the percentage of diseased plants was increased. On the other hand the addition of fishmeal, as an amendment, to the soil led to a remarkable difference in the behavior of selected antagonizing Streptomyces and bacterial species as biocontrol agents separately and collectively, and in induction of resistance in cotton to the pathogenic fungus (R. solani) compared to the non-amended soil. It is also clear that the addition of a mixture of the four Streptomyces and four bacterial species to the soil was found to be most effective against the pathogen and in induction of resistance in cotton plant than each of them alone.

EFFECT OF ACTINOMYCETE AND BACTERIAL SPECIES ON COTTON PLANT GROWTH

Tables (4 & 5) show actinomycetes and bacteria increased the growth measurements compared to the control and each of their mixtures significantly increased all cotton growth measurements (length of root, height of shoot, fresh weight of root, fresh weight of shoot, dry weight of root and dry weight of shoot) compared with each of them alone in both amended and non-amended soils.

PRODUCTION OF IAA AND SIDEROPHORES AND PHOSPHATE SOLUBILIZATION BY ACTINOMYCETE AND BACTERIAL SPECIES

It is shown from table (6) that S. purpureus, A. ramosus and P. putida are characterized by their ability for the production of IAA and siderophore and their ability for phosphate solubilization. S. erumpens and S. microflavus are characterized by their ability for siderophore production and phosphate solubilization, while A. terregens is characterized by its ability for siderophore production only.

RF OF THE ANTIFUNGAL COMPONENTS PRODUCED BY THE SELECTED ACTINOMYCETE AND BACTERIAL SPECIES

Data in tables (7 & 8) show that 5 active components were detected in the culture filtrate of S. erumpens, and 4 active components were detected in the culture filtrates of S. purpureusa, S. aurantiacus and S. microflavus. It is also revealed from the tables that 5 active components were detected in the culture filtrates of all the selected bacterial species. All these active components were found to be effective against R. solani. The active component of Rf 0.13 was detected in the culture filtrates of two Streptomyces and three bacterial species, and the active components of Rf 0.41 was detected in the cultures of three Streptomyces and two bacterial species.

Data in table (9) show the chemical name, chemical formula and molecular weight of the purified active components detected in the culture filtrates of S. aurantiacus and C. pusillum. It was well known that a compound of high molecular weight has a low Rf value and vice versa, therefore, the active components were arranged descendignly according to their Rf values and molecular weight. Figs. (1 & 2) showed the mass spectrum of the purified active components of S. aurantiacus and C. pusillum.

Figure 1

Figure 1: Mass spectrum of the purified antifungal components produced by S. aurantiacus.

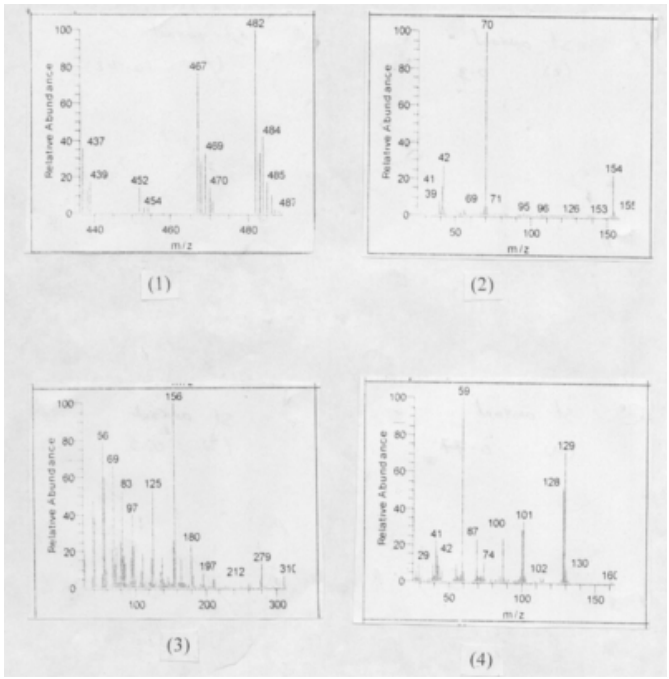
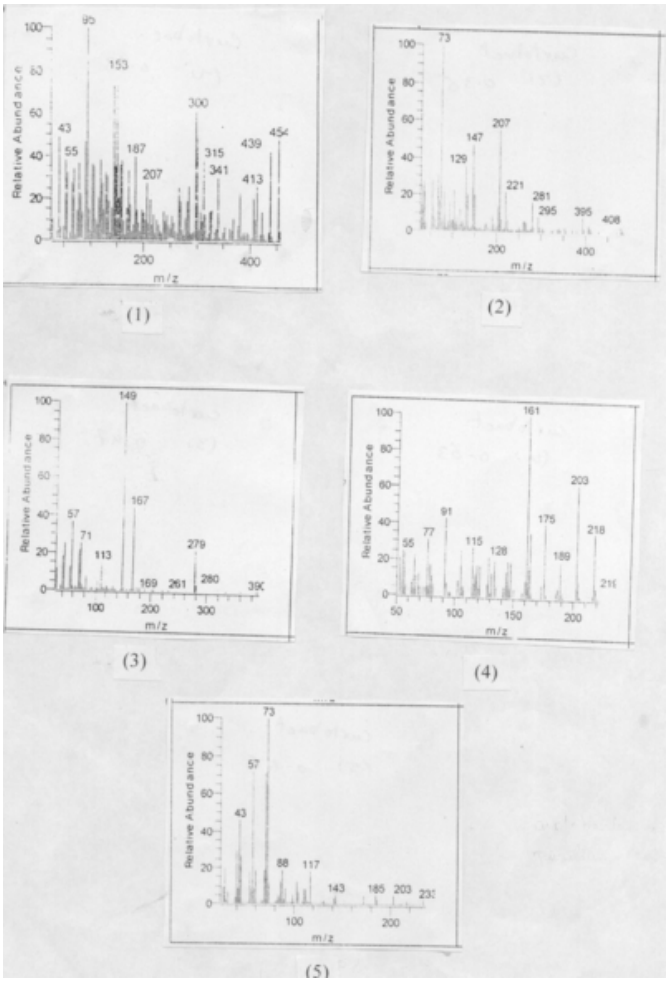


Figure 2

Figure 2: Mass spectrum of the purified antifungal components produced by C. pusillum.



DISCUSSION

In a screening program aimed at the discovery of antifungal substances by the selected actinomycete and bacterial species, these species were found to produce antifungal substances with high inhibitory effect against R. solani. This may be due to their extracellular metabolites which acted on fungal cell membrane altering its permeability ([$_{54}$] or due to suppression of spore germination and/or early stages of mycelial growth of the pathogen [$_{26}$] or the diffusion of antibiotic into the medium [[[[43 and 55]]]]. the inhibitory effect of bacterial species may be due to their antibiosis towards the pathogenic fungi which could be due to the production of antagonistic compounds like siderophores which affect the respiratory system of pathogenic fungi and results in their growth inhibition [$_{20}$>,$_{30}$] or production of antibiotics [$_{23}$].

The reduction in the percentage of seed germination in

presence of R. solani may be due to the role of the pathogen which acts as a causal agent of damping off disease. The pathogen seems to be pathogenic under certain environmental conditions such as temperature or pH, an/ or it may play a secondary role in disease development. It is also possible that R. solani is able to overcome defense mechanisms of cotton plant causing damping off of cotton. These findings are in accordance with that reported by [45].

The use of rhizosphere actinomycetes and bacteria separately or in combination increased the percentage of inhibition of the pathogen. This may be due to antifungal production by the rhizosphere biocontrol agents which may play a significant role in antagonism at the microhabitat level [2].

The ability of plant growth promoting microorganisms to increase plant growth is related in part to antibiosis that occurs in root zones and the subsequent displacement of certain colonizing microorganisms [31].

Increasing the genetic diversity of biological control systems through the use of mixtures of rhizosphere microorganisms may results in treatments that persist longer in the rhizosphere and utilize a wide array of biocontrol mechanisms (e.g. induction of systemic resistance, production of antibiotics and competition for nutrients) under a broad range of environmental conditions [37]. the mechanisms of growth promotion include increased mobilization of insoluble nutrients and subsequent enhanced plant nutrients uptake [36].

A growing body of evidence from various studies indicates that increased resistance may be associated in part with marked metabolic changes in host, including accumulations of hydrolases such as chitinases and -1,3- glucanases with antimicrobial potential, and deposition of structural polymers such as lignin and hydroxyproline-rich glycoproteins, which may be of key importance in the resistance process [52]. strains of fluorescent Pseudomonas species are known to promote the growth of several annual crops by increasing uptake of nutrients such as N and K [5>,48].

The four Streptomyces species and the four bacterial species produce antifungal substances which inhibit the growth of R. solani. This may be due to that cell metabolism of each species under condition of nutritional excess is directed towards the generation of cell mass rather than the production of secondary metabolites and when depletion of key nutrients occurs, it shifts the cell cycle to the stationary phase and signals the transition from primary to secondary metabolism in which the active components are produced. This finding agrees with that reported by Abbaral et al.,[1].

Figure 3

Table 1: Antagonistic activities of actinomycete isolates against .

Actinomycete antagonists	Tested pathogenic fungus (*R. solani*)			
	γ_o	γ	$\Delta\gamma$	% inh.
Isolate No. 1	2.30	1.85	0.45	19.50
Isolate No. 2	2.30	2.20	0.10	4.30
Isolate No. 3	2.30	1.95	0.35	15.00
Isolate No. 4	2.30	2.30	0.00	0.00
Isolate No. 5	2.30	2.30	0.00	0.00
Isolate No. 6	2.30	2.00	0.30	13.00
Isolate No. 7	2.30	1.65	0.65	28.00
Isolate No. 8	2.30	1.45	0.85	36.00
Isolate No. 9	2.30	1.60	0.70	30.00
Isolate No. 10	2.30	2.30	0.00	0.00
Isolate No. 11	2.30	2.00	0.30	13.00
Isolate No. 12	2.30	1.75	0.55	23.00
Isolate No. 13	2.30	1.90	0.40	17.30
Isolate No. 14	2.30	2.30	0.00	0.00
Isolate No. 15	2.30	1.75	0.55	23.00
Isolate No. 16	2.30	2.30	0.00	0.00
Isolate No. 17	2.30	1.55	0.75	32.60
Isolate No. 18	2.30	2.30	0.00	0.00
Isolate No. 19	2.30	1.70	0.60	26.00
Isolate No. 20	2.30	1.85	0.45	19.50
Isolate No. 21	2.30	2.30	0.55	0.00
Isolate No. 22	2.30	1.55	0.75	32.60
Isolate No. 23	2.30	1.20	1.10	47.80
Isolate No. 24	2.30	1.10	1.20	52.00
Isolate No. 25	2.30	1.50	0.80	34.70
Isolate No. 26	2.30	1.70	0.60	26.00
Isolate No. 27	2.30	1.55	0.75	32.60
Isolate No. 28	2.30	1.5	0.80	34.70
Isolate No. 29	2.30	1.45	0.85	36.90
Isolate No. 30	2.30	1.50	0.80	34.70
Isolate No. 31	2.30	0.70	1.60	69.5
Isolate No. 32	2.30	2.00	0.30	13.00
Isolate No. 33	2.30	1.45	0.85	36.90
Isolate No. 34	2.30	2.05	0.25	10.80
Isolate No. 35	2.30	1.60	0.70	30.40
Isolate No. 36	2.30	1.80	0.50	21.70
Isolate No. 37	2.30	1.45	0.85	36.90
Isolate No. 38	2.30	1.65	0.65	28.20
Isolate No. 39	2.30	1.40	0.90	39.00
Isolate No. 40	2.30	1.50	0.80	34.70
Isolate No. 41	2.30	1.25	1.05	45.60
Isolate No. 42	2.30	1.80	0.50	21.70
Isolate No. 43	2.30	1.45	0.85	36.90

Figure 4

Table 2: Antagonistic activities of bacterial isolates against .

Bacterial Antagonists	Tested pathogenic fungus (*R. solani*)			
	γ_o	γ	$\Delta\gamma$	% inh.
Isolate No. 1	2.30	1.40	0.90	39.00
Isolate No. 2	2.30	2.10	0.20	8.60
Isolate No. 3	2.30	1.70	0.60	26.00
Isolate No. 4	2.30	1.87	0.43	18.60
Isolate No. 5	2.30	1.90	0.40	17.30
Isolate No. 6	2.30	1.95	0.35	15.20
Isolate No. 7	2.30	1.75	0.55	23.90
Fluroescent bacteria	2.30	1.85	0.45	19.50

Where: γ_o : Fungal growth radius of a control culture (cm).

γ : Distance of fungal growth in direction in direction of bacteria colony (cm).

$\Delta\gamma = \gamma_o - \gamma$

% inh. : Percentage of inhibition ($\Delta\gamma/\gamma_o$

Figure 5

Table 3: Effect of and bacterial species on the germination of cotton seeds using amended and non-amended soils.

Treatment	Cotton plant					
	Amended soil			Non-amended soil		
	% of seed germination	% of diseased plants	% of control	% of seed germination	% of diseased plants	% of control
Actinomycetes						
1- Untreated (control)				80		
2- Treated:	80					
Pathogen only.		50		37.5	62.5	
S. purpureus + pathogen.	50	25	75	70	37.5	62.5
S. aurantiacus + pathogen.	80	37.5	62.5	70	37.5	62.5
S. erumpens + pathogen.	70	37.5	62.5	60	50	50
S. microflavuis + pathogen.	70	50	50	60	50	50
Mix. + pathogen	60	12.5	87.5	70	37.5	62.5
Bacteria	90					
Untreated (control).				80		
Treated:	80					
Pathogen only.		50		37.5	62.5	
P. putida + pathogen.	50	25	75	80	25	25
A. ramosus + pathogen.	80	37.5	62.5	70	37.5	62.5
A. terregens + pathogen.	70	37.5	62.5	70	37.5	62.5
C. pusillum + pathogen.	70	50	50	60	50	50
Mix. + pathogen.	60	12.5	87.5	70	37.5	62.5

Figure 6

Table 4: The effect of the four species and their mixture on the growth measurements of cotton plant grown in amended and non-amended soils.

Treat.	Length of Root (cm)	Height of Shoot (cm)	Fresh weight of Root (g)	Fresh weight of Shoot (g)	Dry weight of Root (g)	Dry weight of Shoot (g)
A						
C	8.12±(0.15)	9.40±(0.24)	0.14±(0.0036)^a	1.10±(0.0032)	0.10±(0.0025)	0.22±(0.0052)^a
S1	15.02±(0.16)	18.30±(0.23)^b	0.21±(0.0034)	1.50±(0.016)	0.15±(0.0016)^a	0.27±(0.0038)
S2	12.30±(0.081)	17.15±(0.35)^c	0.19±(0.0013)	1.39±(0.0087)	0.15±(0.0037)^a	0.25±(0.0029)
S3	11.08±(0.17)	16.50±(0.40)^c	0.16±(0.0042)^a	1.34±(0.0061)	0.13±(0.0027)	0.22±(0.0049)^a
S4	16.15±(0.13)	19.01±(0.13)^{ab}	0.26±(0.0045)	1.76±(0.0064)	0.34±(0.0033)	0.22±(0.0024)^a
S5	18.18±(0.16)	20.00±(0.10)^a	0.43±(0.0049)	1.98±(0.0021)	0.24±(0.0028)	0.53±(0.0052)
N						
C	5.21±(0.13)	7.25±(0.41)	0.10±(0.0040)	1.01±(0.0026)	0.09±(0.0034)	0.20±(0.0046)^a
S1	10.06±(0.13)^a	16.59±(0.19)^{ac}	0.23±(0.0023)	1.41±(0.0070)	0.15±(0.0044)^a	0.29±(0.0040)
S2	10.03±(0.14)^a	16.10±(0.37)^b	0.20±(0.0027)	1.36±(0.0016)	0.14±(0.0061)^a	0.27±(0.0023)
S3	9.27±(0.31)	5.35±(o.28)^c	0.18±(0.0021)	1.24±(0.0094)	0.12±(0.0025)	0.24±(0.0026)^a
S4	12.27±(0.13)	18.00±(0.31)^a	0.24±(0.0054)	1.61±(0.0065)	0.17±(0.0020)	0.31±(0.0040)
S5	15.28±(0.12)	18.47±(0.27)^a	0.28±(0.0024)	1.77±(0.022)	0.20±(0.0033)	0.34±(0.0032)

A: amended soil N: non-amended soil

C: Untreated (control) S1: *S. aurantiacus* S2: *S. erumpens*

S3: *S. microflavus* S4: *S. purpureus* S5: Mixture

Values with the same letter within a column are not significantly (P>0.05) different according to Fisher's protected LSD test. Results are means of 10 replicates for each treatment. The values in parentheses are the standard error of the mean.

Figure 7

Table 5: Effect of the four bacterial species and their mixture on the growth measurements of cotton plant grown in amended and non-amended soil.

Treat.	Length of Root (cm)	Height of Shoot (cm)	Fresh weight of Root (g)	Fresh weight of Shoot (g)	Dry weight of Root (g)	Dry weight Of Shoot (g)
A						
C	8.12±(0.15)^a	9.40±(0.24)^a	0.14±(0.0036)^c	1.10±(0.0032)^a	0.10±(0.0025)^a	0.22±(0.0052)^a
B1	9.35±(0.16)	12.70±(0.13)	0.16±(0.0027)^a	1.19±(0.0032)	0.11±(0.0030)^a	0.23±(0.0021)^a
B2	7.08±(0.12)	10.00±(0.10)^a	0.13±(0.0026)^b	1.15±(0.0021)^a	0.10±(0.0027)^a	0.21±(0.0023)^b
B3	8.28±(0.11)^a	10.92±(0.16)	0.14±(0.0030)^{bc}	0.15±(0.0047)^a	0.11±(0.0025)^a	0.21±(0.0037)^b
B4	11.27±(0.12)	15.30±(0.33)	0.15±(0.0027)^a	1.39±(0.0040)	0.13±(0.0024)	0.24±(0.0033)^a
B5	13.26±(0.16)	18.88±(0.23)	0.19±(0.0054)	1.48±(0.0050)	0.16±(0.0024)	0.27±(0.0026)
N						
C	5.21±(0.13)^a	7.25±(0.41)	0.10±(0.0040)^c	1.01±(0.0020)	0.09±(0.0034)^b	0.20±(0.0046)^c
B1	5.81±(0.20)^a	9.44±(0.11)	0.13±(0.0033)^b	1.15±(0.0024)^{ab}	0.97±(0.0026)^c	0.20±(0.0026)^c
B2	5.14±(0.19)^a	8.36±(0.26)^a	0.11±(0.0024)^c	1.14±(0.0054)^b	0.08±(0.0021)	0.18±(0.0023)^b
B3	5.32±(0.077)^a	8.68±(o.14)^a	0.12±(0.0029)^c	1.15±(0.0030)^b	0.92±(0.0024)^b	0.19±(0.0021)^b
B4	8.15±(0.15)	10.46±(0.46)	0.14±(0.0024)^{ab}	1.17±(0.0025)^a	0.10±(0.0026)^a	0.21±(0.0024)^a
B5	9.13±(0.15)	11.79±(0.20)	0.15±(0.0024)^a	1.18±(0.0033)	0.11±(0.0023)^a	0.22±(0.0023)^a

A: amended soil N: non-amended soil

B1: *A. ramosus* B2: *A. terregens* B3: *C. pusillum*

B4: *P. putida* B5: Mixture

Values with the same letter within a column are not significantly (P>0.05) different according to Fisher's protected LSD test. Results are means of 10 replicates for each treatment. The values in parentheses are the standard error of the mean.

Figure 8

Table 6: production of IAA and siderophore and phosphate solubilization by rhizosphere and bacterial species.

Selected species	Test		
	IAA production	Siderophore production	Phosphate solubilization
Actinomycete species:			
S. erumpens	-	+	+
S. purpureus	+	+	+
S. aurantiacus	-	+	-
S. microflavus	-	+	+
Bacterial species:			
ramosus	+	+	+
terregenes	-	-	+
C. pusillum	-	+	-
P. putida	+	+	+

Figure 9

Table 7: Rf values of the active components produced by the selected species.

Tested species	R_f of the active components
S. erumpens	0.13
	0.25
	0.41
	0.61
	0.86
	0.08
	0.25
S. purpureus	0.41
	0.81
	0.08
	0.30
S. aurantiacus	0.50
	0.77
	0.13
S. microflavus	0.25
	0.41
	0.81

Figure 10

Table 8: Rf values of the active components produced by the tested bacterial species.

Tested species	R_f of the active components
A. ramosus	o.13
	0.25
	0.52
	0.63
	0.81
A. terregens	
	0.19
	0.30
	0.41
	0.58
C. pusillum	0.86
	0.13
	0.36
	0.47
	0.63
P. putida	0.86
	0.13
	0.36
	0.47
	0.69
	0.91

Figure 11

Table 9: Chemical name, chemical formula and molecular weight of the purified active components of the culture filtrates of and .

Chemical name	Chemical formula	Molecular weight
S. aurantiacus:		
2,3-dihydro-3-methoxywisthacnistin acetate.	$C_{23}H_{38}O_9$	578
2-bromo-3,3',5,5'-tetra(1-butyl)-biphenyl.	$C_{28}H_{41}Br$	456
5-hydroxy-2,2-dimethyl-5m6-bis(2-oxo-1-propyly)-1-cyclohexanone.	$C_{14}H_{22}O_4$	254
Butanedioic acid,methyl-,dimethyl ester.	$C_7H_{12}O_4$	160
C. pusillum:		
Tossendanin.	$C_{30}H_{38}O_{11}$	574
1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-hexasiloxane.	$C_{12}H_{38}O_5Si_6$	430
1-2,benzendicarboxylic acid, bis(2-ethylhexyl)ester.	$C_{24}H_{38}O_4$	390
2,6-ditertbutyl-4-hydroxy methylphenol.		
Ethyl-2-hydroxybutyrate.	$C_{15}H_{24}O_2$	236
	$C_6H_{12}O_3$	132

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