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Streptomyces MUTANTS AND PROTOPLAST REGENERATION AS TOOLS FOR BIOCONTROL OF PLANT PATHOGENIC FUNGI.

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ABSTRACT

Sixteen Streptomyces cultures,i.e, four wild type strains, eleven mutants and one regenerated protoplast isolate were tested for their abilities to inhibit one or more of three local isolates of the plant pathogenic fungi, *Fusarium solani, Rhizoctonia solani* and *Sclerotium rolfsii,* which cause root rot disease.

Bacillus subtitles was used to measure the antibacterial antibiotic activity of *Streptomyces* strains and mutants. All tested cultures were antibacterial antibiotic producers except R1, G2 and *Str. abus*. Mutant R4 was the best antibacterial producer.

The sixteen cultures were tested for their antifungal activity against *Fusarium solani*. All of them inhibited *F. solani* growth after three days of incubation. After seven days, the antifungal activities of two-strains, *Str. albus* and *Str. Griseovirdis was* lost. Antifungal activities of eight mutants increased by increasing incubation period. Mutants R2, R4 and G4 were the higher antifungal activity mutants against *F. solani*. The best producer was R2 mutant, either after three or seven days of incubation.

Two strains, *Str. albus* and *Str. griseovirdis* were inactive against *Rhizoctonia solani,* after three and seven days of incubation. The activities of seven cultures were decreased after seven days of incubation. Mutants R2, VT and GR were the higher antifungal activity after seven days of incubation. G1 mutant was the best antifungal activity against *Rhizoctonia solani* after three days of incubation.

All tested cultures showed antifungal activities against *Sclerotium rolfsii*. Three cultures, R1, *Str. albus* and *Str. griseovirdis,* lost their activities after seven days of incubation. Three mutants G1, G2 and G3 isolated from *Str. griseus,* were higher antifungal activities against *R. solani*. The best antifungal activity was G2 after three and seven days of incubation.Since, G2 was inactive as antibacterial antibiotic producer so in some cases, it is advantage to use such mutant as a biocontrol strain.

On the basis of the above results, *S. rolfsii* showed to be very sensitive to most of the Streptomyces tested cultures. Mutant R2 proved to be the best in its antifungal activity against two out of the three tested plant pathogenic fungi. GR, which was selected after protoplast regeneration, has a remarkable antifungal activity. It was one of three higher antifungal activities against *R. solani* after seven days of incubation.

Keywords: *Streptomyces*, Mutants, Biocontrol, Fungi, Root rot, Protoplast, *F. solani, R. solani, S. rolfsii.*

INTRODUCTION

Fungal diseases cause millions of dollars worth of crop damage all over the world However, the methods used during the last 50 years which depended mainly upon the use of chemicals for the controlling of such enemies resulted, unfortunately, in a plenty of problems of which appeared genetically resistant breads of the fungi themselves, the pollution of the yield itself and even the environment, with such chemicals. These reflect many harmful hazards on the human health, elimination of the biological enemies naturally found, the chemical contamination and the maintained residue of such chemicals into the land. Also, environmental concerns and development

of resistance in target populations have reduced the availability of effective fungicides.

For such hazardous effects it appeared the importance of using biological control methods, through which all the foregoing pollution effects could be avoided and the balance between both harmful pests and its biological enemies could be returned. Recently attention has focused on the development of biocontrol techniques (Crowe and Olsson 2001).

Nonetheless, the vast array of antimicrobial molecules produced by diverse soil microbes' remains as a reservoir of new and potentially safer biopesticides. For these and other reasons, a heightened interest in so-called biological control or biocontrol (i.e., the use of natural microorganisms or their products to limit attack and damage by phytopathogens) has arisen (Kang *et al.* 1998).

However, in the last few years there have been relatively few studies of bacteria, especially actinomycetes, applied to seeds and roots for the purpose of controlling diseases. Biocontrol may operate through antibiosis or competition for space or nutrients in the rhizosphere (El-Tarabily *et al.* 1997).

Streptomycetes are gram-positive mycelial bacteria, were studied primarily for their ability to produce a large portion of the naturally occurring antibiotics. The effects of streptomyces strains come from several complex factors. The microbe deprives pathogenic fungi around plant roots. In soil, they are able to synthesize a wide range of various enzymes and metabolites, which inhibit pathogens growth (Walter and Schrempf 1995).

To demonstrate the role for antibiotics in biocontrol, mutants lacking production of antibiotics or over-producing mutants have been used by Whipps (2001). Alternatively, the use of reporter genes or probes to demonstrate production of antibiotics in the rhizosphere is becoming more commonplace, isolation and characterization of gene(s) clusters responsible for antibiotic production has now been achieved. It was generally accepted that mutation and subsequent arising of variant organisms is a spontaneous process that generates a pool of genetically different individuals in a population (Wery *et al.* 2001).

In addition, protoplast regeneration proved to be a good tool for inducing genetic variations. During regeneration, protoplast size increased many times. DNA replication occurs at a stage before individual genomes become partitioned into regenerated hyphae-possibly enough replication to produce hundreds, or even thousands, of genomes. Genetic variation occurs during these rounds of replication (Hopwood 1981).

In the present study, sixteen cultures either wild types, mutants or isolates after protoplast regeneration, were selected and tested under *in vitro* conditions for their abilities to inhibit one or more of the three local plant pathogenic fungi, which cause root rot diseases.

MATERIALS AND METHODS

Strains

Bacterial and fungal strains used in this study are listed in Table 1.

Table (1): Bacterial and fungal strains.

Culture	Relevant features	Source or References
Streptomyces		
R1, R2 ,R3 and R4	Spontaneous mutants isolated from *Str. Rimosus* ATCC 10970*	This study
Str. Venezuelae DSM 40727	Wild-type	DSM
VY, VS and VT	Spontaneous mutants isolated from *Str. Venezuelae* DSM 40727.	This study
Str. griseus	Wild-type	NRC [1]
G1, G2, G3 and G4	Spontaneous mutants isolated from *Str. Griseus.*	This study
GR	Isolated from *Str. griseus* after protoplast regeneration	This study
Str. albus NRRL 3917	Wild-type	NRRL
Str. griseoviridis NRRL 2427	Wild-type	NRRL
Plant pathogenic fungi		
Fusarium solani	Wild-type	NRC[2]
Rhizoctonia solani	Wild-type	NRC[2]
Sclerotium rolfsii	Wild-type	NRC[2]
Antibiotics testers		
B. subtilis NRRL B-453	Wild-type	NRRL
Candida lipolytica Y-6	Wild-type	NRC [1]

*** *Str. rimosus* ATCC 10970 was tested for their antibacterial antibiotic and antifungal activity against plant pathogenic fungi in previous work, Khalil (1990) and Ashour *et al.* (1995).**

ATCC, American Type Culture Collection, USA.
NRRL, National Regional Research Laboratory, Egypt.
DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany.
NRC[1] kindly provided by Microbial Chemistry Dept., National Research Center, Egypt.
NRC[2] kindly provided by Plant Pathology Dept., National Research Center, Egypt.

Isolation of mutants:

Morphological mutants appeared during propagation steps were picked up and subcultures several times and tested for stability of antibiotics production.

Media

All media and solutions are conducted according to Khalil (1990) and Tayel *et al.* (2001).

Protoplast isolation and regeneration:

Str. griseus protoplast preparation, regeneration and media were carried out according to Tayel *et al.* (2001)

Antibacterial bioassay

Petri dishes 16-cm in diameter each containing 50 ml of CM medium, were each inoculated with four Streptomyces tested cultures. Plates were incubated at 28 °C for four days Two ml of over-night *B subtilis* culture as a tester organism was placed on the surface of each plate. Plates were incubated over-night at 28 °C and clear zones of inhibition were measured, according to Khalil (1990) and Tayel *et al.* (2001).

Antifungal Bioassay

Bioassay Petri dishes 16-cm in diameter containing 50-ml medium were used. Each one was inoculated with four Streptomyces tested cultures. The three local plant pathogenic fungi under investigation were inoculated each in Petri dish and after incubation, 5-mm agar discs bearing fungal mycelia were transferred to the medial of bioassay plates. Control plates inoculated with 5-mm agar discs fungal mycelia only. Plates were incubated at 28 °C and clear zone diameters were measured, according to Ferreira *et al* (1991).

RESULTS AND DISCUSSION

1- *Streptomyces griseus* protoplast

Protoplasting was carried out according to Tayel *et al.* (2001). About 99% protoplasts were isolated from *Str. griseus* mycelia after treatment. Figure 1 showed *Str. griseus* mycelia and the isolated protoplasts. Protoplasts were plated on regeneration medium. Large numbers of regenerated colonies were isolated and tested for their antifungal activity using *Candida lipolytica* as a tester organism (data not shown). Only one regenerated isolate, GR, was selected as the best antifungal producer compared with *Str. griseus* parental strain.

It has been known from some time that genetic variances usually occur during protoplast regeneration. However, Okanishi *et al.*, (1974) examined the regenerating unfused *Str. griseus* and *Str. venezuelae* protoplasts using phase-contrast microscope Each protoplast swells to a relatively enormous size from a body with a diameter of 1-2 µm to a structure up to 40 µm in diameter. Volume increased many thousand times, assuming that the sphere is not a disk, from the periphery of which several regenerated hyphae grow out; these eventually develop into the sporulating colony. Hopwood (1981) added that considerable DNA replications occurs at a stage before individual genomes become partitioned into regenerated hyphae to produce hundreds, or even thousands, of genomes. Recombination presumably occurs during these rounds of replication.

2- Streptomyces Mutants

A large number of morphological mutants were isolated through propagation and practical studies. Mutants proved to be stable with higher antifungal activity using *Candida lipolytica* as a tester, were selected. Four mutants were isolated form *Streptomyces rimosus* ATCC 10970. However, *Str rimosus* wasn't used in this study because it was tested before, Ashour *et al.* (1995) It showed low antifungal activity against *Fusarium solani and Rhizoctonia solani* strains. Three mutants were isolated from *Str. venezuelae* DSM 40727 and four from *Str griseus* All selected mutants are present in Fig 2.

The four mutants R1, R2, R3 and R4 that were isolated from *Streptomyces rimosus* ATCC 10970 are non-sporulation mutants with brown colony color and produce brown pigments while *Str. rimosus* is a very rich sporulation, white colony and brown pigment.

Fig. 1. *Str. griseus* cells (A) and protoplasts (B), using phase contrast microscope.

Mutants VY, VS and VT are poor spore producers with yellow, light brown and brown colony colors respectively The three mutants are pigment producers but VT produced a remarkable amount of black pigment, which effected colony color appearance as a black However, the wild type strain, *Str. venezuelae* DSM 40727, has a very rich sporulation, white colony and black pigment.

Mutants of *Str. griseus*, G1, G2 and GR a regenerated protoplast, are non-spores producers, with brown colonies and produced brown pigments However, mutant G3 is poor sporulation with deep brown color and brown colony. *Str. griseus* is a very rich spores producer with white colony color and brown pigment producer.

All the different available strains of Streptomyces were tested for their antifungal activity. Furthermore, two other wild type strains, *Str. albus* NRRL 3917 and *Str. griseoviridis* NRRL 2427 were used in addition to *Str. venezuelae* DSM 40727 and *Str. griseus*. These wild type strains are very rich in spore production and their colonies have white color Fig. 2.

An explanation for spontaneous mutants occurring and its roles in action, it was recognized by Wery *et al.* (2001) that DNA in living organisms is not static. The process of continuous mutation of DNA enables adaptation to changing environments and it is a prerequisite for evolution. It was generally accepted that mutation and subsequent arising of variant organisms is a spontaneous process that generates a pool of genetically different individuals in a population under nonselective conditions, from which under selective conditions the individual(s) with beneficial mutation(s) will originate

3- Antibacterial activities

Antibacterial productions play an important role in inhibition or effecting other organisms or bacteria in soil, which effect positively or negatively plant growth. Sixteen selected cultures were tested for their antibacterial activity using *B. subtilis* as a tester organism. Results in Fig. 3 showed that, all mutants isolated from *Str. rimosus* except R1 were higher antibacterial producers than it whether after three or seven days of incubation. *Str. rimosus* antibacterial antibiotic production was measured in previous work. (data not shown).

Three mutants isolated from *Str. venezuelae* were also higher antibacterial antibiotic producers than the wild type. VS mutant produce about 1.6 times more than wild type after three or seven days of incubation Furthermore, the regeneration protoplast isolate GR and G1 mutant were higher antibacterial antibiotics producer than the wild type strain, *Str. griseus*.

On the other hand, the three cultures, R1, G2 and *Str. albus*, were inactive, i.e, no antibacterial activities were detected after three or seven days of incubation.

Antibacterial production of six tested cultures were decreased after seven days of incubation, while six cultures showed increase in their antibacterial activity after seven days of incubation. The antibacterial activity one mutant, G3, did not effect by incubation period more than three days

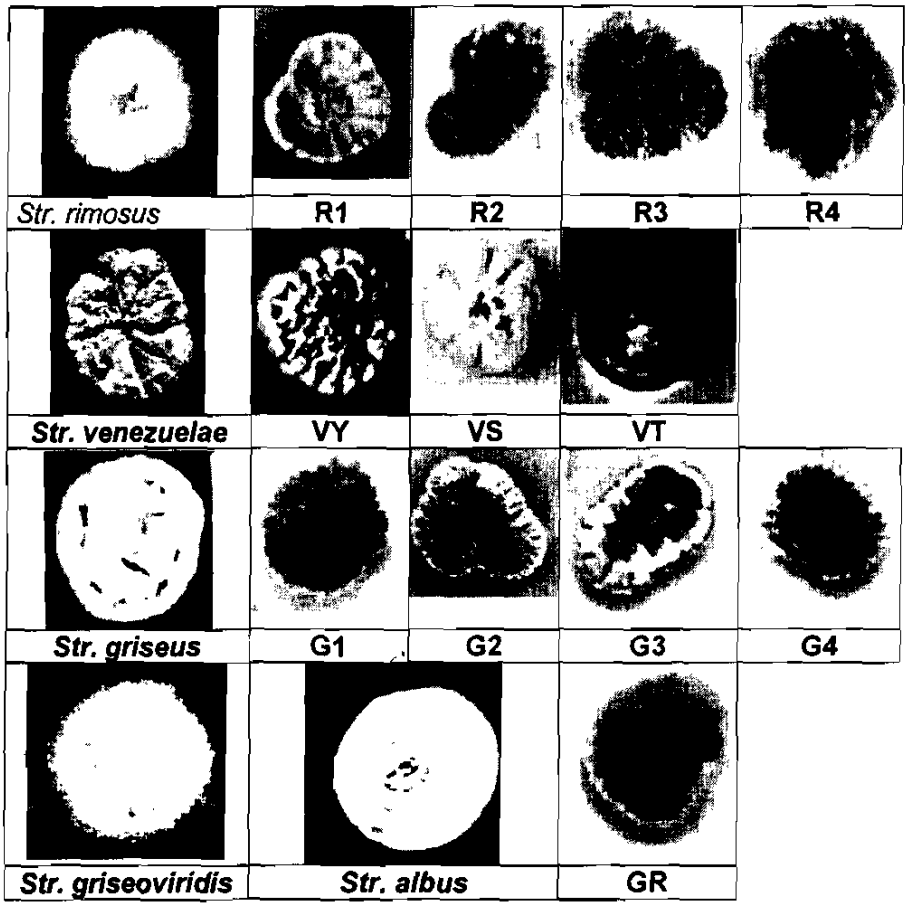


Fig. 2 : Morphology of *Streptomyces* strains and mutants Colonies.

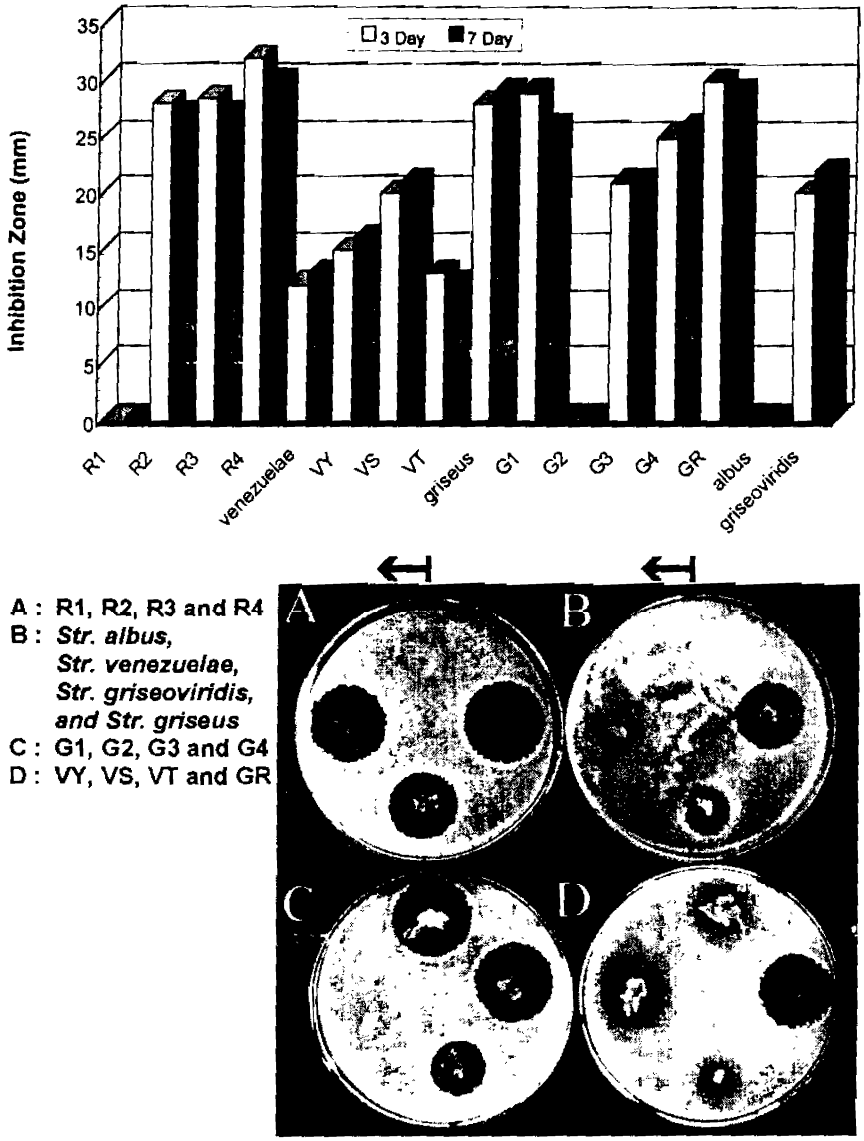


Fig.3 . Antibacterial antibiotic production of sixteen *Streptomyces* strains and mutants, *Bacillus subtills* was used as a tester organism.

The higher producer cultures were R4, GR and G1. The best antibacterial antibiotic producer mutant R4, which produce Oxytetracycline (OTC) Khalil (1990), was isolated from *Str. rimosus*. It reached its maximum activity after three days. However, oxytetracycline (OTC) is commercially an important antibiotic produced by *Streptomyces rimosus*. The cluster genes of 30 kb in size flanked by two resistance genes lie in 600 kb from one end of the 8 Mb linear chromosome. *Streptomyces rimosus* is genetically unstable and some spontaneous mutants carry large-scale DNA rearrangements involving the OTC-cluster, which might account for its frequent spontaneous amplification and deletion. Class II mutants isolated by Pandza *et al.* (1997) showed large deletions that recover the whole cluster, whereas class III mutants showed reiteration of the cluster resulting in higher production and resistance to, OTC.

4- Biological Control (Antifungal activity)

Eleven mutants and one regenerated colony in addition to wild types *Str. albus* NRRL 3917, *Str. griseoviridis* NRRL 2427, *Str. venezuelae* DSM 40727 and *Str. griseus* strains were selected. They were tested for their abilities to inhibit one or more of three local strains of plant pathogenic fungi, *F. solani, R. solani* and *S. rolfsii*, which cause root rot disease.

a- Fusarium solani

All selected cultures were tested for their ability to inhibit the *Fusarium solani* growth, which case wilt root disease. Results are present in Figure 4. Antifungal antibiotics production of *Str. venezuelae* and its mutants were nearly the same after 3 days, but after 7 days, the wild type and VY were the same in their antifungal antibiotics while the other *Str. venezuelae* mutants were less activity against *F. solani* compared with wild type strain.

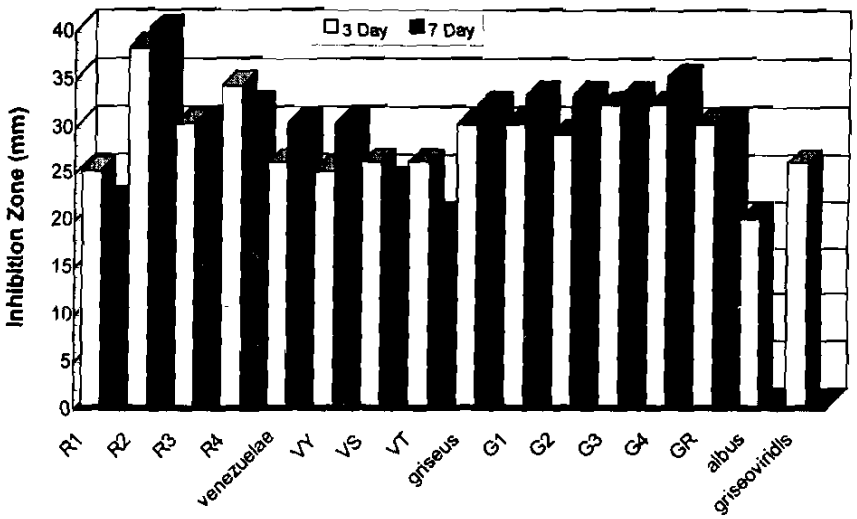
Str. griseus, G4 mutant and GR were the same antifungal activity against *F. solani*. G3 and G4 mutants showed higher antifungal activity than wild type after three days of incubation. However, all mutants isolated from *Str. griseus* were higher activity against *F. solani* after seven days of incubation.

Two cultures, out of sixteen were lost their antifungal activity after 7 days of incubation while it was increased in eight mutants. Six mutants showed decreasing in their ability to inhibit *Fusarium solani* growth after seven days incubation. Only two mutants kept the same level of antifungal activities.

Two mutants proved to be higher producers after 3 days and two mutants after 7 days of incubation. However, R2 mutant was the best producer mutant after 3 or 7 days of incubation. It was isolated from *Str. rimosus*, which produced rimocidin as antifungal antibiotic.

b- *Rhizoctonia solani*

Mutants isolated from *Str. Venezuelae* (Fig. 5) were less antifungal activity than it against *R. solani* after three days of incubation. VS mutant was higher antifungal activity than wild type while the other mutants showed the same wild type activity after seven days of incubation.



A : R1, R2, R3 and R4
B : *Str. albus, Str. venezuelae, Str. griseoviridis, and Str. griseus*
C : G1, G2, G3 and G4
D : VY, VS, VT and GR

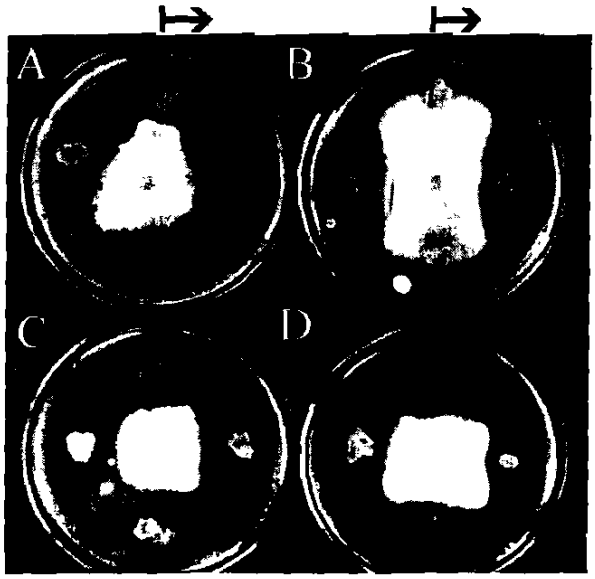


Fig. 4. Antifungal productivity of sixteen *Streptomyces* strains and mutants against the growth of *Fusarium solani*.

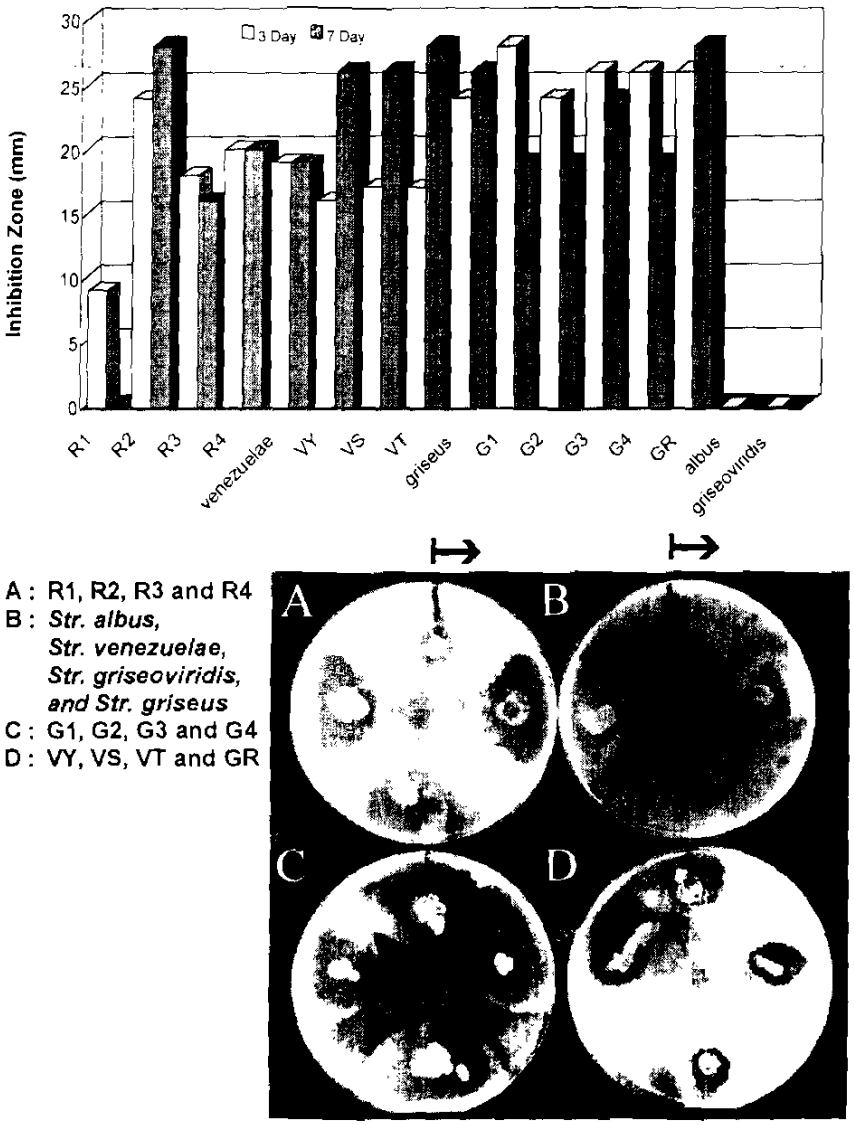


Fig. 5. Antifungal productivity of sixteen *Streptomyces* strains and mutants against the growth of *Rhizoctonia solani*.

One mutant G2, showed the same activity like *Str. griseus* against *R. solani* The other mutants including regenerated isolate GR were higher antifungal activity than the wild type, after three days of incubation. The situation was changed after seven days of incubation, all mutants were less activity than the wild type except regenerated isolate GR which was higher antifungal activity against *R. solani.*

Two cultures, *Str. albus* and *Str. griseoviridis,* out of sixteen were inactive against *Rhizoctonia solani.* Results presented in Figure 5 also showed that, one mutant R1 lost its antifungal activity after seven days of incubation. Antifungal activity of two mutants against *R. solani* was the same after 7 days. Antifungal activities of 5 mutants increased while activities of seven mutants decreased after seven days of incubation.

Mutants R2, VT and GR were higher antifungal producers against *R. solani* after incubation for seven days.However, G1 mutant was the best antifungal producer, it was reached maximum activity after three days of incubation.

c- Sclerotium rolfsii

After three days of incubation, all sixteen cultures inhibited the growth of *S. rolfsii.* Results presented in Figure 6 showed that, all *Str. venezuelae* mutants were lower activity than it after three days of incubation, against *S. rolfsii.* However, after seven days of incubation, all mutants showed the same wild type activity except VS, which proved to be higher antifungal activity than wild type.

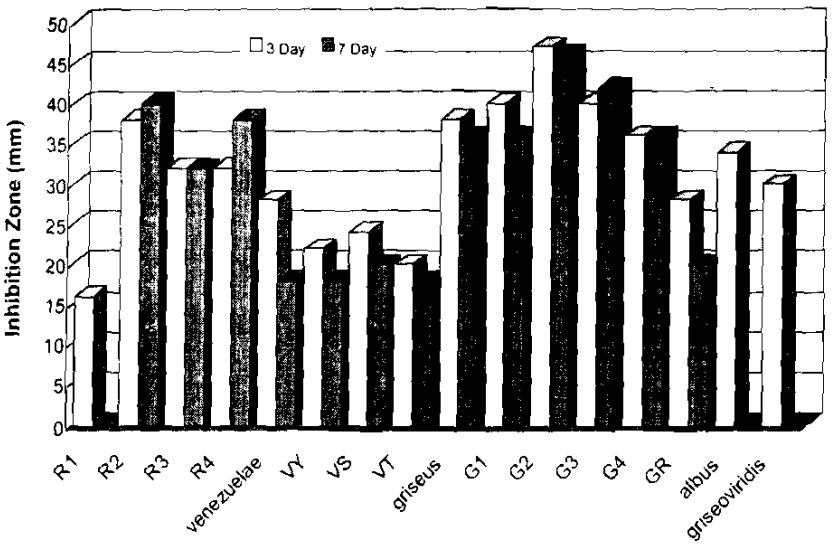
G2 mutant and GR showed less activity than *Str. griseus* while the other three mutants were higher antifungal activity against *S. rolfsii,* after three days of incubation. Three mutants showed the same wild type activity against *S. rolfsii,* while GR was less activity after seven days of incubation. However, VS was higher antifungal producer than wild type.

Two wild types, *Str. albus, Str. griseoviridis* in addition to one mutant, R1, lost their antifungal activities after seven days of incubation. Antifungal activities of eleven cultures decreased, while activities of three mutants were increased after seven days of incubation. Incubation period did not effect the ability of mutants R3 and G4 to inhibit *S. rolfsii* growth.

On the other hand, three mutants G1, G2 and G3 were the higher antifungal producer. G2 mutant that isolated from *Str. griseus,* proved to be the highest antifungal activity against *S. rolfsii.* Although the antifungal activity of G2 decreased after seven days of incubation, but it was still the best antifungal producer culture whether after 3 or 7 days.

Although G2 was inactive as antibacterial antibiotic producer, but it prove to be the best mutant as antifungal activity against *S. rolfsii.* It will be an advantage to be use it as a biocontrol mutant for *S. rolfsii* fungus without affecting other than bacteria in rhizosphere.

From these results it was clear that mutants not only differ in their morphology than their wild types but they also differ in their activities against plant pathogenic fungi. Moreover, mutants isolated from the same wild type differ in their antifungal activities during incubation periods to reach their maximum activity and production stability.



A : R1, R2, R3 and R4
B : *Str. albus*, *Str. venezuelae*, *Str. griseoviridis*, and *Str. griseus*
C : G1, G2, G3 and G4
D : VY, VS, VT and GR

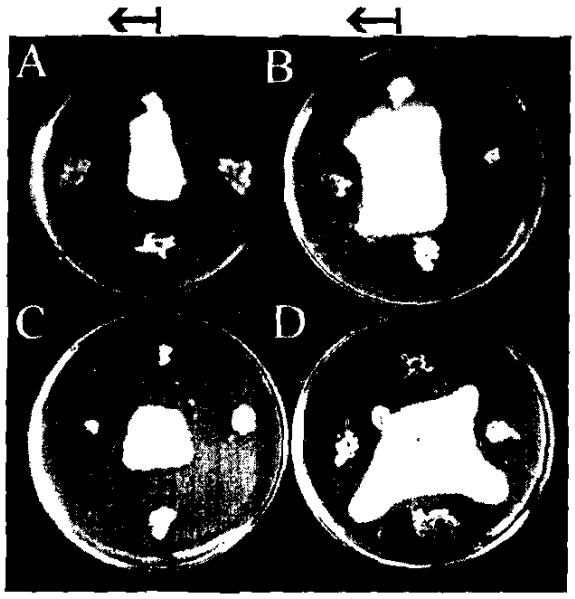


Fig. 6. Antifungal productivity of sixteen *Streptomyces* strains and mutants against the growth of *Sclerotium rolfsii*.

On the basis of the above mentioned results, although *Str. griseoviridis* is a biocontrol strain, but it has low or inactive antifungal activity against the local plant pathogenic fungi under investigation. Mutants isolated from *Str. rimosus* and *Str. venezuelae* showed high levels of antifungal activity. Mutant R2 proved to be the best in its antifungal activity against two out of the three tested plant pathogenic fungi. However, *S. rolfsii* sowed the highest level of sensitivity to the most Streptomyces tested cultures.

GR, which was selected after protoplast regeneration as a higher antifungal producer against *C. lipolytica*, has, in addition to a remarkable antifungal activity, it was one of the three higher antifungal producers against *R. solani* after seven days of incubation.

The previous results of Malina *et al.* (1985) indicated that, the regeneration of protoplasts was able to induce different types of genetic variants. From *Str. incarnatus* a variant was isolated, which yield 27 fold more antibiotic in comparison with the original. Khalil (1990) used protoplast fusion as a tool for the genetic improvement of antibiotic production in *Streptomyces rimosus*. He obtained 183 different biotypes showing wide range in the production of oxyteteracycline from which some positively exceeded their parental isolates by about 70 %.

In this study, spontaneous mutations play an important role in controling the growth of the plant pathogenic fungi. However, Fischer *et al.*, (1997) reported that, most of the spontaneous mutations were due to large chromosomal deletions removing the ends of the linear chromosome. The chromosome becomes circular and is then even more unstable than the linear chromosome. Schmid *et al.* (1999) proposed that, genetic instability is a widespread phenomenon in the genus *Streptomyces*: mutants acted in various phenotypic properties like morphological differentiation, secondary metabolism or antibiotic resistance arise at frequencies between 0.1% and 1% of colony-forming spores.

Another interesting explanation for the previous results proposed that, Streptomyces wild-type chromosome is linear in all examples studied by Volff and Altenbuchner (1998). The ends of the chromosome or telomeres consist of terminal inverted repeats of various sizes. The chromosome is very unstable and undergoes very large deletions spontaneously at rates higher than 0.1% of spores, [Güne *et al.* (1999) and Hopwood (1999)]. The spontaneous mutation in gene blocked biosynthesis of the antimicrobial agent was also studied by Duffy and Défago (2000) in the model biocontrol strain *Pseudomonas fluorescens* CHA0. Spontaneous mutants had altered abilities to utilize several carbon sources and to increase medium pH compared with the wild type.

Furthermore, in previous work, *Str. rimosus* and 31 of its mutants were selected by Ashour *et al* (1995). Bioassay technique was applied to test their antifungal effects against fungal strains causing wilt, root rot diseases and leaf blight disease. Six mutants proved to be higher active against the plant pathogenic fungal growth comparing with the wild-type activity. in percentages which reached in one case 188%.

Different microorganisms other than Streptomyces were also used for biocontrol. Strains of the bacterium *Pseudomonas fluorescens* that are

antagonistic to *R. solani* were isolated by Crowe and Olsson (2001) and evaluated as biocontrol agents. A soil isolate of *Pseudomonas fluorescens* (BL915) was shown to be an effective antagonist of *Rhizoctonia solani*. which induced damping-off of cotton. Investigation of the biological basis of this antagonism revealed that the strain produces pyrrolnitrin, a secondary metabolite known to inhibit *R solani* and other fungi. Mutants of strain BL915 that did not produce pyrrolnitrin and did not suppress damping-off of cotton by *R. solani* were generated by exposure to N-methyl-N (prm1)-nitro-N-nitrosoguanidine, Hill *et al* (1994).

To day, search for soil bacteria is becoming a tool to use microorganisms as potential biocontrol agents for several plant diseases. The purpose of Ciampi and Tewari (1990) research was to detect bacteria biological control with special, *R. solani*, *Pythium* spp., and *Fusarium* spp., which case seedling damping-off. Out of three hundred forty-one bacterial cultures isolated, only 16 inhibited fungal growth, 7 showed the same effects against *R. solani* and 9 showed uneven effects. Identification showed that 3 bacterial belonged to *Bacillus* spp., 4 to green fluorescent *Pseudomonas* spp. and 2 were *Streptomyces* spp.

Antibiotic produced by bacteria particularly pseudomonads plays an important role in biocontrol. It seems to be closely regulated by a two-component system involving an environmental sensor (presumably a membrane protein) and a cytoplasmic response factor, Keel and Défago (1997). Mutation in either gene has similar multiple effects on antibiotic production.

Moreover, *in vitro* antibiotic activity of 15 *T. harzianum* isolates was assayed against 10 isolates of five different soilborne fungal plant pathogens: *Aphanomyces cochlioides*, *Rhizoctonia solani*, *Phoma betae*, *Acremonium cucurbitacearum*, and *Fusarium oxysporum* f. sp. radicis lycopersici. Similarities between levels and specificities of biological activity and the numerical characterization groupings are both discussed in relation to antagonist-specific populations in known and potential biocontrol species, Grondona *et al.* (1997).

Biocontrol modes of action include, inhibition of the pathogen by antimicrobial compounds (antibiosis); competition for colonization sites and nutrients supplied by seeds and roots; induction of plant resistance mechanisms; inactivation of pathogen germination factors present in seed or root exudates; degradation of pathogenicity factors of the pathogen such as toxins; parasitism that may involve production of extracellular cell wall-degrading enzymes, for example, chitinase and ß-1,3 glucanase that can lyse pathogen cell. Whipps (1997). Indeed, for some biocontrol agents, different mechanisms or combinations of mechanisms may be involved in the suppression of different plant diseases, (Whipps 2001).

The obtained results in the present study revealed that G2 mutant could be considered as a promising one for biocontrol approach. G2 was inactive as antibacterial antibiotic. In some cases, especially beneficial groups (N_2 fixing bacteria). it is an advantage to use such mutant as a biocontrol strain since it will not affect the bacterial balance in rhizosphere.

Based on the results of this study, investigations on possible control measures have been initiated. Emphasis is being giving to clarify whether the ability of biocontrol agents are stable or transient and the consistency of their performance must be improved. Taking into consideration this well, requirements research in many diverse areas, since biological control is the culmination of complex interactions among the host, pathogen (s) . antagonist (s) and environment, are needed.

REFERENCES

Ashour, E. H.; K. M .A. Khalil and A .A. Ismail (1995). *Streptomyces rimosus* a proposed biofungicide for the biocontrol of some plant pathogenic fungi. 1st, International Conf. of Pest control, Mansoura, Egypt, Sept. 301-310.

Ciampi, L. and J.P. Tewari (1990). Evaluation of soil microorganisms with inhibitory activity against *Rhizoctonia solani* causal agent of the damping-off of canola. *Arch Biol Med Exp (Santiago),* 23(2):101-12.

Crowe, J. D. and S. Olsson (2001). Induction of Laccase Activity in *Rhizoctonia solani* by Antagonistic *Pseudomonas fluorescens* Strains and a Range of Chemical Treatments. Appl. Environ. Microbiol., 67 (5): 2088-2094.

Duffy, B. K. and G. Defago (2000). Controlling Instability in *gacS-gacA* Regulatory Genes during Inoculant Production of *Pseudomonas fluorescens* Biocontrol Strains. Appl. Environ. Microbiol., 66 (8):3142-3150.

El-Tarabily, K. A.; G. Hardy; K. Sivasithamparam; A. M. Hussein and Kurtboke (1997). The potential for the biological control of cavity-spot disease of carrots, caused by *Pythium coloratum*, by streptomycete and non-streptomycete actinomycetes. New Phytologist 137, 495–507.

Ferreira, J. H. S.; F.N. Mathhee and A. C. Thomas (1991). Biological control of *Eutypa lata* on grapevine by an antagonistic strain of *B. subtilis*. Phytopathology, 81:283-287.

Fischer, G.; B. Decaris and P.Leblond (1997). Occurrence of deletions, associated with genetic instability in *Streptomyces ambofaciens*, is independent of the linearity of the chromosomal DNA. *J Bacteriol* 179, 4533-4558.

Grondona, I.; R. Hermosa; M. Tejada; M. D. Gomis; P. F. Mateos; P. D. Bridge; E. Monte; and I. Garcia-Acha (1997). Physiological and biochemical characterization of *Trichoderma harzianum*, a biological control agent against soilborne fungal plant pathogens. Appl. Environ. Microbiol., 63(8): 3189-3198.

Güne, G.; B. Smith and P. Dyson (1999). Genetic instability associated with insertion of IS*6100* into one end of the *Streptomyces lividans* chromosome. *Microbiology*, 145: 2203-2208.

Hill, D. S.; J. I. Stein; N. R. Torkewitz; A. M. Morse ; C. R. Howell; J. P. Pachlatko; J. O. Becker and Ligon, J. M. (1994). Cloning of Genes Involved in the Synthesis of Pyrrolnitrin from *Pseudomonas fluorescens* and Role of Pyrrolnitrin Synthesis in Biological Control of Plant Disease. Appl. Environ. Microbiol., 60 (1) :78-85.

Hopwood, D. A (1981). Genetic Studies With Bacterial Protoplasts, Ann Rev Microbial , 35:237-72

Hopwood, D. A. (1999). Forty years of genetics with *Streptomyces*: from *in vivo* through *in vitro* to *in silico*. Microbiology, 145: 2183-2202.

Kang, Y.; R. Carlson; W. Tharpe and M. A. Schell (1998). Characterization of Genes Involved in Biosynthesis of a Novel Antibiotic from *Burkholderia cepacia* BC11 and Their Role in Biological Control of *Rhizoctonia solani*. Appl. Environ. Microbiol., 64 (10):3939-3947.

Keel, C.; and G. Defago (1997). Interactions between beneficial soil bacteria and root pathogens: mechanisms and ecological impact. In: GangeAC, Brown VK, eds. Multitrophic interactions in terrestrial system. Oxford: Blackwell Science, 27–47.

Khalil, K. M. A. (1990). Protoplast fusion in some prokaryotics. Ph. D. Thesis. Ain Shams Univ., Faculty of Agriculture.

Malina, H.; C. Tempete; and R. G. Maalka (1985). Enhanced sinefungin production by medium improvement, mutagenesis and protoplast regeneration of *Str. incarnatus* NRRL 8099. J. Antibiotics, 38: 513-525

Okanishi, M.; K. Susuki and Umezowa, H. (1974). Formation and reversion of *Streptomyces* protoplast: cultural condition and morphological study. J. Gen. Microbiol., 80: 389-400.

Pandza, K.; G. Pfalzer; J. Cullum and D. Hranueli (1997). Physical mapping shows that the unstable oxytetracycline gene cluster of *Streptomyces rimosus* lies close to one end of the linear chromosome. Microbiology 143 (5):1493-501.

Schmid, E.; C. Büchler and J. Altenbuchner (1999). AUD4, a new amplifiable element from *Streptomyces lividans*. *Microbiology*, 145: 3331–3341.

Tayel, A. A.; K. M. A. Khalil; F. M. I. Badawy and R. N. Abbas (2001). Genetic Imrovement Of *Amycolatopsis Mediterranei* for Rifamycin SV Antibiotic Production Using Mutation nd rotoplast usion. Egypt. J Genet. Cytol., 30:87-102.

Volff, J. N. and J. Altenbuchner (1998). Genetic instability of the Streptomyces chromosome. *Mol Microbiol*, 27(2):239-46.

Walter, S and H. Schrempf (1995). Studies of *Streptomyces reticuli cel-1* (Cellulase) Gene Expression in *Streptomyces* Strains, *Escherichia coli*, and *Bacillus subtilis*. Appl. Environ Microbiol., 61(2) :487–494.

Wery, J.: B. Hidayat; J. Kieboom and J. A. M De Bont (2001). An Insertion Sequence Prepares *Pseudomonas Putida* S12 For Severe Solvent Stress. J. Bio. Chem., 276(8): 5700 – 5706.

Whipps, J. M. (2001). Microbial interactions and biocontrol in the rhizosphere. Journal of Exper. Bot., 52 : 487-511.

Whipp.s J.M (1997). Developments in the biological control of soil-borne plant pathogens. Adv. in Botan. Res, 26: 1–134.

طافرات الإستربتومايسس والبروتوبلاست مستعيد الجدار كوسائل للمكافحة الحيوية للفطريات الممرضة للنبات

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اختبرت قدرة ١٦ مزرعة استربتومايسس (اربعة طرز برية واحدى عشر طافرة وواحدة عزلت بعد استعادة البروتوبلاست للجدار) على تثبيط نمو واحد او اكثر من ثلاثة فطريات محلية مسببة لمرض عفن الجذور فى النبات. تلك الفطريات هى ريزوكتونيا سولانى و سكليروتيم رولفزيى و فيزاريم سولانى .

إستخدمت سلالة باسيلس ساتلس لقياس قدرة سلالات وطافرات الإستربتومايسس على إنتاج المضادات الحيوية البكتيرية . كانت كل المزارع المختبرة منتجة للمضادات الحيوية البكتيرية فيما عدا الطافرتين R1 و G2 وسلالة إستربتومايسس البس وكانت الطافرة R4 هى افضل الطافرات إنتاجية للمضاد الحيوى البكتيرى .

إختبرت الستة عشر مزرعة لنشاطها كمضادات لفطر فيوزاريم سولانى حيث كانت كلها ذات تأثير مثبط لة بعد ثلاثة أيام تحضين. بينما فقدت سلالتين هذه القدرة بعد سبعة أيام و زاد النشاط المثبط للفطر فى ثمانى طافرات. وكانت أفضل الطافرات نشاطا كمضادات فطرية ضد فيوزاريم سولانى هى R2 و R4 و G4 لكن كان أفضلهم على الاطلاق هى الطافرة R2 سواء بعد ثلاث او سبعة ايام تحضين .

لم تبديا السلالتان إستربتومايسس ألبس و إستربتومايسس جريزيوفيرديز اى نشاط يذكر ضد فطر الريزوكتونيا سولانى سواء بعد ثلاثة او سبعة ايام تحضين . هذا وقد قل نشاط سبع مزارع بعد سبعة ايام تحضين .بينما كان للطافرات R2 و VT و GR افضل نشاط تثبيطى بعد سبعة ايام تحضين فى حين كانت الطافرة G1 هى افضلهم نشاطا كمضاد فطرى ضد ريزوكتونيا سولانى وذلك بعد ثلاثة ايام تحضين .

كان لكل المزارع المختبرة نشاط كمضاد فطرى ضد سكليروتيم رولفزيي, فى حين فقدت ثلاث (مزارع إستربتومايسس ألبس و إستربتومايسس جريزيوفيرديز وR1) قدرتها بعد سبع ايام تحضين. بينما كانت الطافرات G1 و G2 و G3 والمعزولة من إستربتومايسس جريزيس اعلى المزارع نشاطا كمضاد فطرى مثبط للسكليروتيم رولفزيى. وكانت الطافرة G2 افضل نشاطا كمضاد فطرى سواء بعد ثلاث او سبعة ايام تحضين . ومما يذكر ان الطافرة G2 ليس لها إى نشاط كمضاد بكتيرى مما يعد ميزة فى بعض الاحيان عند إستخدامها فى المقاومة الحيوية كمضاد فطرى وحتى لايفقد التوازن الطبيعى للبكتيريا النافعة فى التربة.

أظهرت النتائج السابقة ان سكليروتيم رولفزيي اكثر الفطريات حساسية لمعظم مزارع الاستربتومايسس المختبرة. والطافرة R2 افضل المزارع المختبرة كمضاد فطرى لأثنين من الثلاث فطريات المختبرة والممرضة للنبات. وكانت GR والناتجة عن البروتوبلاست مستعيد الجدار واحدة من افضل ثلاثة كمنتج للمضادات الفطرية للريزوكتونيا سولانى بعد سبعة أيام تحضين.