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Interspecific *Streptomyces* Protoplast Fusants as Biological Control Agents

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ABSTRACT

The present study aimed to construct superior *Streptomyces* bacteria for improvement antibiotic production with mutagenic agents treatments and protoplast fusion techniques. Two *Streptomyces* sp. were applied in this study *i.e. S. fradiae* KCC-5-0133 and *S. endus* KCC-5-0213. The obtained interspecific fusants were used as biological control agents against *Fusarium oxysporum, Alternaria solani* and *Streptomyces scabies*. Treatment of wild type strains with different concentrations of MNNG for one hour showed that, out of 79 isolates obtained from *S. fradiae*, 17 isolates excelled the wild type in antibiotic production. When MNNG was applied with *S. endus* at different concentrations and the same exposure time, 21 isolates out of 83 ones exceeded its parental strain. Wild type strains treated with EMS showed that out of 100 isolates from *S. fradiae*, 21 isolates proved to be higher than the parental strain in antibiotic production. Eighteen isolates, out of 100 were selected randomly from *S. endus*, for their highest in antibiotic production. Protoplast fusion technique was applied between the selected survivors following the application of MNNG and EMS treatments. The highest and the lowest efficient antibiotic producer isolates, as well as, the parental strains were used. Nine crosses were carried out; the first cross involved the two parents (*S. fradiae* KCC-5-0133 and *S. endus* KCC-5-0213). The second to the fifth crosses involved four isolates selected from the survivors resulted from treatment both *S. fradiae* and *S. endus* with MNNG mutagen. Meanwhile the sixth to ninth crosses were performed between four isolates selected from the survived isolates following treatment of *S. fradiae* and *S. endus* with EMS mutagen. In general, interspecific protoplast fusion in the present study produced some new constructed isolates; Twenty out of 26 fusants lost their ability of inhibition zone formation with the tester strains. All of the six effective fusants were similar to the parental strains since they were effective with *M. luteus* tester strain. Moreover, three out of six interspecific protoplast fusants proved to be promising ones in biological control of plant pathogens. Two different pathogenic fungi (*A. solani* and *F. oxysporium*) and *Streptomyces scabies* which cause common scab in potato were used to determine the biological control efficiency of *Streptomyces* strains as well as their fusants. The obtained results clearly exhibited that only three out of the obtained 26 interspecific fusants proved to be highest effective ones for inhibition the growth of both *A. solani* and *F. oxysporium* fungi. The three effective isolates have the same or exceeded the effect of the parental strain *S. endus* KCC-5-0213. On the other hand, these effective interspecific fusants proved to be highly effective for *Streptomyces scabies* control as the other parental strain (*S. fradiae* KCC-5-0133) do.

Key words: *Streptomyces*, mutagenic treatments, antibiotic production, protoplast fusion, biological control.

INTRODUCTION

Streptomycetes are gram-positive mycelial soil bacteria that produce a wide variety of secondary metabolites. Many of these metabolites have important application as antibiotics or other useful compounds in human medicine and in agriculture. Genetic manipulation of producing organism can be used to improve the efficiency of production or development a novel antibiotic made by combinatorial biosynthesis, but the important elements in productivity is the availability of precursors and cofactors (Butler *et al.*, 2002).

So many studies were conducted previously in order to obtain genetic variability in different *Streptomyces sp.* using chemical and/or physical mutagenic agents, e.g., Moile and Buttner (2000) and Lanoot *et al*. (2005). The potentialities studied in these mutants induced were antibiotic production; antibiotic resistance or aerial mycelia and spores formation.

The protoplast formation, intra and/or interspecific fusion and fusants regeneration are important processes, and they are a major step following genetic manipulations which can improve antibiotic production. Different investigations were conducted and reported dealt with *Streptomyces sp.* protoplast fusion (Nazari *et al.*, 2005).

The combination of streptomycetes abundance in soil and their ability to produce a wide variety of biologically active substances suggested that this group of organisms may play a significant role in plant-microbe and microbe-microbe interactions, so that Streptomycetes have been used as a biological control agent against a variety of plant pathogens (Yu *et al.*, 2007).

The present study was conducted in Genetics and Microbial Biotechnology Lab., Genetic Dept., Agric. Fac.; Kafr El-Sheikh University, EGYPT and amid to construct effective *Streptomyces* genotypes to biological control harmful pathogenic microorganisms.

MATERIALS AND METHODS

Microorganisms

(A) Streptomyces strains

Streptomyces fradiae KCC-5-0133 and *Streptomyces endus* KCC-5-0213, were kindly provided by

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Prof.Dr.S.Ogata, Microbial Genetics Division, Institute of Genetics Resources, Faculty of Agriculture, Kyushu University, Fukuoka, Japan.

(B) Tester strains

Pseudomonas putida (B-13) and *Micrococcus luteus* NRR B-287 (B-87) were provided by Dr. A.A. Khattab, Applied Microbial Genetics Lab., Genetic and Cytology Dept., National Research Center, Dokki, Cairo, Egypt.

(C) Pathogenic strains

Fusarium oxysporium, Alternaria solani and *Streptomyces scabies* were used as pathogenic agents to determine the efficiency of *Streptomyces* strains and their fusants as a biocontrol agent. These strains were provided by Dr. Gabr El-kot & Ms. Nagwa El-khateb, Plant Pathology Dept., Agriculture Fac., Kafr EL-Sheikh University, Egypt.

Media

A lot of media were used to carry out this work , *i.e*., Luria medium (Sambrook *et al.*, 1989) for culturing and maintaining the bacterial tester, Rye flakes agar (Ogata *et al.*, 1981) was used for fermentation and maintaining the *Streptomyces* strains, Treptone soya broth (TSB) for activating *Streptomyces* strains before protoplast fusion, Yeast extract-malt extract (YEME) (Kieser *et al.*, 2000) for growing *Streptomyces* strains for protoplast fusion,R_2YE medium (Thompson *et al.*, 1980) for regeneration protoplast fusants and Potato Dextrose Agar (PDA) medium for culturing and maintaining pathogenic fungi in biological control experiment, Nutrient Agar (Difco Manual, 1977) for culturing and maintaining *Streptomyces scabies* in biological control experiment.

Mutagens

1- N-methyl-N-nitro-N-nitrosoguanidine (MNNG), from Koch-light Laboratory (LTD) England.

2- Ethyl methane sulphonate (EMS) from Sigma Company.

Antibiotics

Different antibiotics and their concentrations were applied to determine the level of resistance or sensitivity to be used as additional selective genetic marker (s) for protoplast fusion. Tetracycline (TC) 50 mg/ml and Kanamycin (Km) 5mg/ml were used for protoplast fusion.

Reagents and buffers

Protoplast formation and fusion buffers were used according to Hopwood and Wright (1978).

Methods

1- Determination of the antibiotic productivity was carried out according to Augustin *et al*. (2004).

2- The mutagencity was conducted as in Delic *et al.*, (1970). According to the survival curves (data not shown) *S. fradiae* was treated with 1, 2 and 3 mg MNNG/ml while *S. endus* was treated with 4, 5 and 6 mg/ml**.** Meanwhile both parental strains were treated with 10, 20 and 50 µl EMS /ml.

3- Protoplast formation and fusion were done according to Kieser *et al.* (2000).

4- In bioassay of biological control efficiency, *Streptomyces* parental strains and their fusants were grown in rye flakes liquid medium, for 5 days at 28^{o}C for 120 rpm, then culture centrifuged for 20 min at 6000 rpm to separate the bacterial filtrate**s** which sterilized using 0.45 µm membrane filters. Adequate volumes of filtrates 1.50, 2.25 and 3.75 ml were added to adequate volumes, 13.5, 12.75 and 11.25 ml of PDA medium, respectively, to obtain concentrations of 10, 15 and 25% (v/v) of filtrates. The PDA treated plates (5cm) were inoculated in the centre with 8-day old mycelial discs (6 mm diameter each) of *F-oxysporium* and *A. solani* with three replicates. The negative control for each filtrate treatments were applied using rye flakes liquid medium without any inoculations. Growth diameter was measured when negative control growth fulls the plates area. Regarding *S. scabies,* the filtrate agar well-diffusion method was used with 100 µl/well from culture isolate filtrate and the inhibition zones were determined.

RESULTS

Mutagenic treatments and antibiotic productivity

(A) N-methyl-N-nitro-N-nitrosoguanidine (MNNG)

Bacterial cell suspensions of *S. fradiae* KCC-5-0133 and *S. endus* KCC-5-0213 were treated with MNNG mutagen. The data presented in Table (1) appeared a dose-response for cell survival of both treated species. It showed that the survival percentages decreased sharply by increasing the concentration of MNNG with the constant of exposure time. The survival percentage of S. *fradiae* was 23.99 at 1 mg, and dropped down to zero following the highest concentration (3 mg). In addition, the total count of colonies which were grown after 1 and 2 mg/ml of MNNG for one hour were isolated to determine their antibiotic productivity.

Moreover, data in Table (1) illustrates also the effect of different concentrations of MNNG on survival percentages with *S. endus*. The obtained results clearly showed that number of counted colonies and of course survival percentages decreased rapidly by increasing the mutagen concentration. The survival percentages was 30.30 after one hour with the lowest dose (4 mg/ml), and reduced to 1.72% following the highest dose (6 mg/ml). Random fifty single colonies were isolated

following the first MNNG treatments to determine their antibiotic productivity, in addition to all survived colonies following the second and third concentration treatments.

(B) Effect of Ethyl methane sulphonate (EMS)

The original culture of *S. fradiae* KCC-5-0133 and *S. endus* KCC5-0213 were treated with EMS. Bacterial cell suspension of *S. fradiae* and *S. endus* were treated with different concentrations of EMS. Data in Table (2) clearly showed a dose-response in survival percentages which decreased by increasing mutagen concentrations.

Fifty random single colonies from both *S. fradiae* & *S. endus* were isolated from the treatments of 10 and 20 µl/ml to determine their antibiotic productivity.

Antibiotic productivity after mutagenic agents treatments

To determine the effect of mutagenic agents treatments on the antibiotic productivity of the selective isolates in comparison with their wild type strains; the tester strain *P. putida* was applied with *S. fradiae* and its treated derivatives. *Micrococcus luteus* was adopted with both of *S. endus* and its treated derivatives. (*P. Putida* proved to have resistance for *S. endus* ones).

(A) Antibiotic productivity of S. fradiae following mutagenic treatments

Data in Table (3) presents the antibiotic productivity of S. *fradiae* following treatments with 1 and 2 mg MNNG/ml, as well as, 10 and 20 µl EMS/ml.

Seventy seven isolates survived following treatment with one mg MNNG/ml for one hour and their antibiotic efficiency was determined. Seventeen isolates proved to produce effective antibiotic higher than the original strain. The obtained results showed that 32 isolates lost some of the antibiotic efficiency since it exhibited smaller inhibition zone than its parental strain do. On the other hand, 28 isolates behaved typically as its wild type strain. Following application of two mg MNNG/ml, only two colonies were obtained. One of them showed the same efficiency; and the other produced lower efficient antibiotic than *S. fradiae* strain.

The results show also that out of the randomly selected 50 isolates following the treatment with 10 µl EMS/ml; only five isolates exceeded their *S. fradiae* parental strain. Meanwhile 39 lost some of its antibiotic efficiency and six were as like as their parental strain. Regarding the treatment with 20 µl EMS/ml, 13 isolates exceeded their *S. fradiae* strain; 10 lost some of its antibiotic efficiency and 27 were the same as their parent.

(B) Antibiotic productivity of S. endus following mutagenic treatments

Data in Table (4) presents the antibiotic productivity of S. *endus* following treatments with four, five and six mg MNNG/ml, as well as, 10 and 20µl EMS/ml.

The data summarized antibiotic production efficiency of randomly selected 50 isolates following 4 mg

Table (1): Survival percentages of *Streptomyces*.sp treated with MNNG for one hour.

S. fradiae			*S .endus*		
Concentrations (mg/ml)	**No. of counted colonies**	**Survival (%)**	**Concentrations (mg/ml)**	**No. of counted colonies**	**Survival (%)**
0	321	100.00	0	406	100.00
1	77	23.99	4	123	30.30
2	2	0.62	5	26	6.40
3	0	0.00	6	7	1.72

Table (2): Survival percentages of *Streptomyces*.sp treated with EMS for one hour.

S. fradiae			*S. endus*		
Concentrations (µl/ml)	**No. of counted colonies**	**Survival (%)**	**Concentrations (µl/ml)**	**No. of counted colonies**	**Survival (%)**
0	1130	100.00	0	607	100.00
10	510	45.13	10	410	67.54
20	234	20.71	20	374	61.61
50	0	0.00	50	0	0.00

Table (3): Efficiency of antibiotic production of survived *Streptomyces fradiae* KCC-5-0133 following treatment with MNNG & randomly selected 50 ones following EMS treatments against *Pseudomonas putida* (B-13).

MNNG				**EMS**			
Dose (mg/ml)	**No. of Tested Colonies**	**Efficiency (%)**	**No. of isolates**	**Dose (µl /ml)**	**No. of Tested Colonies**	**Efficiency (%)**	**No. of isolates**
0	---	=100	----	0	---	=100	----
		=100	28			=100	6
1	77	>100	17	10	50	>100	5
		<100	32			<100	39
		=100	1	20		=100	27
2	2	>100	0		50	>100	13
		<100	1			<100	10

treatment. The results showed that 14 isolates exhibited antibiotic efficiency higher than the wild type strain. Meanwhile following dose 5 mg/ml treatment only seven isolates out from 26 survived ones produced antibiotic higher than the wild type isolate. Following treatment with 6 mg/ml none of the seven survivors exceeded their parental strain.

Data showed that out of the randomly selected 50 isolates following the treatment with 10 µl EMS/ml; 15 isolates exceeded their *S. endus* original strain. Meanwhile 30 lost some of its antibiotic efficiency and five were as like as their parent. Regarding the treatment with 20 µl EMS/ml, 10 isolates exceeded their *S. endus* original strain; 24 lost some of its antibiotic efficiency and 16 were the same as their parent.

Protoplast fusion and antibiotic productivity

To study the effects of interspecific protoplast fusion on antibiotic production, the selected isolates and their parents were subjected to different antibiotics in order to determine an additional selective marker(s). Results clearly showed that the effect of MNNG and EMS were only on antibiotic productivity which appeared as regulatory effects, but no effect were observed with antibiotic response and no marker were found to carry out intraspecific protoplast fusion. So that, this study was carried out interspecific protoplast fusion using Tetracycline (50 mg/ml) and Kanamycin (5 mg/ml).

The preparation of *Streptomyces* protoplasts was done according to lysozyme treatments. The whole cell wall digestion was achieved following incubation at 30^{o}C for 60 min. as shown in Figure (1) which described the protoplasting steps of *S. fradiae* and *S. endus*, as well as, its derivatives isolates following MNNG and EMS treatments.

Equal volumes (500 µl) of both parental protoplast suspensions were used for protoplast fusion and nine crosses were carried out as shown in Table (5).

The data in Table (5) appeared the first cross (ϕ x ϕ) involved the two parents (*S. fradiae* KCC-5-0133 and *S. endus* KCC-5-0213). The second to the fifth crosses involved four isolates selected from the survived isolates following treatment both *S. fradiae* and *S. endus* with MNNG, while the sixth to ninth crosses were performed between four isolates selected from the survived isolates following treatment of *S. fadiae* and *S. endus* with EMS mutagen. The parental strains cross gave nine non effective interspecific fusants. Although no fusants were obtained following crossing the low MNNG treated isolate of *S. fradiae* with both *S. endus* isolates (crosses 4 and 5), however using the high *S. fradiae* isolate produced two fusants when crossed to

Table (4): Efficiency of antibiotic production of randomly selected 50 isolates of *Streptomyces endus* KCC-5-0213 treated with 4mg MNNG and the survivors following the other doses and randomly selected 50 ones following each EMS treatments against *Micrococcus luteus* (B-287).

MNNG				EMS			
Dose (mg/ml)	**No. of tested tolonies**	**Efficiency (%)**	**No. of isolates**	**Dose (µl/ml)**	**No. of tested colonies**	**Efficiency (%)**	**No. of isolates**
0	---	=100	----	0	---	=100	----
		=100	17			=100	5
4	50	>100	14	10	50	>100	15
		<100	19			<100	30
		=100	9	20		=100	16
5	26	>100	7		50	>100	10
		<100	10			<100	24
		=100	2				
6	7	>100	0				
		<100	5				

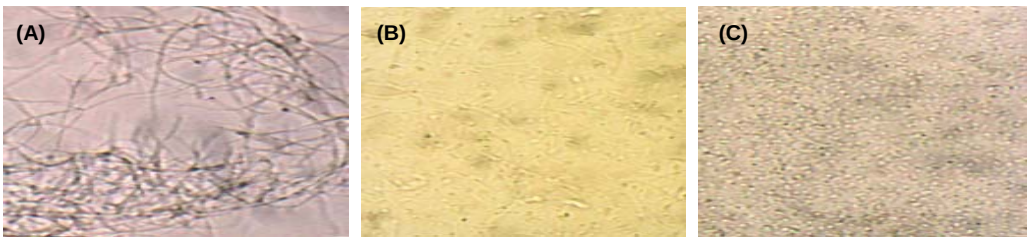

Figure (1): Photomicrographs of *Streptomyces* protoplasts (B and C) in comparison with normal cells (A).

Table (5): Protoplast fusion crosses between both *S. fradiae*, *S. endus* and their derivatives.

Crosses	Cross 1 ϕ x ϕ	Cross 2 H1 x H2	Cross 3 H1 x L2	Cross 4 L1x H2	Cross 5 L1 x L2	Cross 6 H3 x H4	Cross 7 H3 x L4	Cross 8 L3 x H4	Cross9 L3 x L4
No. of obtained fusants	9 *N.EF.	2 **EF.	1 EF.	0	0	3 2 N. EF. +1 EF.	0	2 N.EF.	9 7 N.EF. +2 EF.

H1 = The highest isolate of *S .fradiae* after MNNG treatment, H2 = The highest isolate of *S.* endus after MNNG treatment, H3 = The highest isolate of *S. fradiae* after EMS treatment, H4 = The highest isolate of *S.* endus after EMS treatment, L1 = The lowest isolate of *S. fradiae* after MNNG treatment , L2 = The lowest isolate of *S .enduse* after MNNG treatment, L3 = The lowest isolate of *S .fradiae* after EMS treatment, L4 = The lowest isolate of *S. enduse* after EMS treatment, * Not effective fusant, **Effective fusant

the high *S. endus* isolate and one fusant when crossed to the lowest *S. endus* one (crosses 2 and 3 respectively). An opposite trend was detected when the EMS treated isolates were crossed. The lowest EMS treated *S. fradiae* isolates produced two fusants when crossed to the highest *S. endus* one (cross 8) and nine fusants when crossed to the lowest *S. endus* isolate (cross 9). Two fusants out of these nine ones proved to be highly effective against *M. luteus* than their parental strains. Moreover, no fusants were obtained when the highest *S. fradiae* was crossed to the lowest *S. endus* isolate (cross 7). Meanwhile three fusants were obtained following the highest by highest *S. fradiae* and *S .endus* isolates crossing (cross 6). The effective one was selected as promising one since it exceeded its parents.

Efficiency of *Streptomyces* strains and their fusants in biological control

Two different fungal strains (*A. solani* and *F. oxysporium*) and *Streptomyces scabies* which cause common scab in potato were used as a sample to determine the efficiency of *Streptomyces* strains in biological control, in addition to their fusants. The effect of culture filtrates of parental *Streptomyces* strains and the obtained fusants on growth inhibition average of the pathogenic strains was detected.

The growth diameter average (X) was measured after the growth of (*A. solani* and *F. oxysporium*) fungi on PDA medium (three replicates), with three concentrations (v/v) of culture filtrate (10, 15 and 25%), then, the standard deviation (S.D) was determined to measure the deviation from the (X). The antagonistic efficiency was calculated as follows:

$$\text{Antagonistic efficiency} = \frac{C - I}{C} \times 100$$

C = Growth diameter average of negative control (without filtrate).

I = Growth diameter average following filtrate addition.

Figures (2 and 3) present the antagonistic effects of the two original strains *S. fradiae* and *S. endus* (plates No. 2&3) compared with the effects of the three promising interspecific fusants (plates 4, 5 & 6) and three from the non effective ones (plates 7, 8 & 9). The negative control (untreated) shown in plate No.1.

Data presented in Figures 2 and 3 showed that the original strain *S. fradiae* did not have any antagonistic effect with the two fungi as same as the negative control. Meanwhile *S. endus* gave different efficiencies increased gradually, *i.e.*, 63.64, 76.59 and 84.09 percent by filter concentration increasing (10, 15 and 25%) with *A. solani* , while this parent exhibited efficiencies (94.77, 96.17 and 100.00 percent) following the application of 10,15 and 25% of culture filtrate with the fungal *F. oxysporium*.

In cross 6, the third fusant (fusant 6-3) was higher than the effective parent with antagonistic efficiency by filtrate concentration increasing i.e., 84.09, 86.36 and 100 percent with *A. solani,* respectively, while the efficiency were 84.77, 95.46 and 100.00 percent with *F. oxysporium*. In cross 9, fusant (No. 9-3 and 9-4) showed high antagonistic efficiency in comparison with the original parents. The antagonistic efficiency with *A. solani,* were 80 percent in the first concentration in fusant No. 9-3, while in the rest concentrations in fusant 9-3 and all three concentrations in fusant 9-4, the

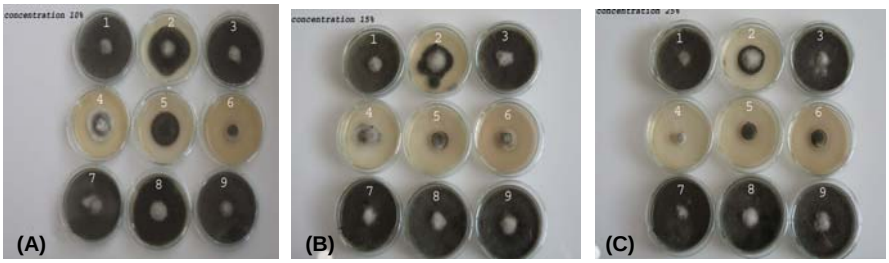

Figure (2): Effect of culture filtrates of parental strains(2-*S.endus* , 3-*S.fradiae*) ,the highest fusant isolates (4-fusant 6-3, 5- fusant 9-3, 6-fusant 9-4)and the lowest fusant isolates (7-fusant 1-1, 8-fusant 9-5, 9-fusant 9-7) at different concentrations (10,15 and 25%) on growth inhibition of *A .solani* compared to the negative control (1).

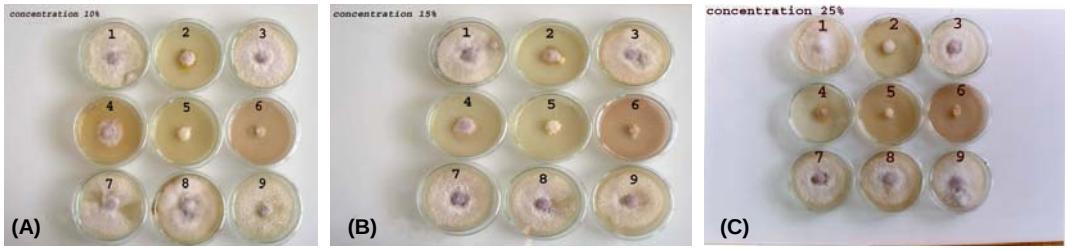

Figure (3): Effect of culture filtrates of parental strains (2-*S.endus* , 3-*S.fradiae*) ,the highest fusant isolates (4-fusant 6-3, 5- fusant 9-3, 6-fusant 9-4) and the lowest fusant isolates (7-fusant 1-1, 8-fusant 9-5, 9-fusant 9-7) at different concentrations (10, 15 and 25%) on growth inhibition of *Fusarium oxysporium* compared to the negative control (1).

efficiency were 100 percent. Fusants No. 9-3 and 9-4 were the highest effective ones in their efficiency with *F. oxysporum*, since the antagonistic efficiency were 100 percent in all three concentrations.

When the effect of culture filtrates of the original parents and their promising fusants were applied against *Streptomyces scabies* through the filtrate agar well-diffusion method, Data presented in Figure (4) showed that the first parent (*S. fradiae*) exhibited inhibition zone formation (25mm) when 100 µl of its culture filtrate were used, while the second parent (*S. endus*) didn't have any inhibitory effect. Two out of the three effective interspecific fusants exceeded the effective parent (*S. fradiae*) as shown in plates No (s). 6, 7 & 8 (25, 31 and 27 mm, respectively) The selected non effective three interspecific fusants (wells No. 3, 4 & 5) did not exhibited any clear zone which indicate that they lost completely their inhibitory effect.

Figure (4): Photograph of *Streptomyces scabies* inhibition zone resulted from culture filtrate from the two parental strains (1- *S. endus* 2-*S.fradiae*), the lowest fusants (3-fusant 1-1, 4- fusant 9-7, 5-fusant 9-9) and the highest fusants (6-fusant 6-3, 7-fusant 9-3, 8-fusant 9-4).

DISCUSSION

Several previous studies reported that the mutagenic treatments of different *Streptomyces* species showed little enhancement of antibiotic productivity and efficiency. Since Baeshin *et al.* (1992) isolated four high efficient NTG treated *S. rimosus* while the rest were either non antibiotic producers or lower than the original strain. Moreover Cheng *et al.* (2001) found that non of thousands survivors following NTG and U.V treatments of *S. hygroscopicus* showed improved antibiotic productivity since the level of antibiotic production depended on both the type and position of amino acid substitution in ribosomal protein. Dairi *et al.* (1995) isolated higher titer producing mutants derived from the parental strain *S. aureofaciens* NRRL 3203 as a result of NTG treatments. Chen *et al.* (1998) obtained similar results from *S. noursei* var. xichangensis FR-5 treated with UV and NTG which indicated that the genetic background of the treated *Streptomyces* species influenced the improvement of the antibiotic (s) productivity.

It could be concluded that, interspecific protoplast fusion in the present study produced some new isolates lost antibiotic productivity, since 20 out of 26 fusants lost their ability of inhibition zone formation ability with the tester strains. All of the six effective fusants were similar to the original parents (*S. fradiae & S. endus*) since they were effective against *M luteus* tester strain. The three highly effective interspecific fusants (one from cross 6 and two from cross 9) were highly recommended to be applied as biological control agents.

The above results are in agreement with those obtained by Hopwood and Wright (1978) and Baltz (1978) since they conducted that the genomes fragmented and exchange of genes occurs as a result of protoplast fusion.

The obtained results of biological control assay were in parallel with those reported by Okamura *et al.* (1989) since they isolated TT-strain after protoplast fusion between *S. fradiae* and *S. antibioticus* which was active on a range of gram-positive bacteria. On the other hand the obtained results showed good harmony with Malanicheva *et al.* (1992) since they carried out protoplast fusion between two strains from *S. fradiae* and after regeneration of the protoplasts, no strain producing antibiotics differing the original strain were isolated since all isolates were unstable and lost their ability to synthesis the antibiotic. Also, Agbessi *et al.* (2003) isolated two recombinants after protoplast fusion at intraspecific level in *S. melanosporofaciens* EF-76. Strain FP-54 exhibited higher antagonistic activities against *S. scabies* and *Phytophthora fragariae var. rubi* 390 since it was shown to produce two antimicrobial compounds at lest that were absent in the parents.

In conclusion the present study enhanced the efficiency of some *Streptomyces* mutagenic treated isolates as well as some interspecific protoplast fusants for antibiotic production. Moreover, three interspecific protoplast fusants proved to have excellent antagonistic effect against *A. solani*; *F. oxysporium* and *Streptomyces scabies* pathogenic organisms. This growth inhibition phenomenon will lead to reduce the environmental pollution as a result of limitation of fungicides and bacteriocides application.

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إنتاج مندمجات الاستربتوميسس البروتوبلاستية بين النوعية كعوامل فعالة للمكافحة الحيوية

جمعة على بهجت الفاضلى، على احمد محمد أبو شوشة، مدحت رمضان أمين ريحان
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الملخص العربي

أجريت هذه الدراسة بهدف الحصول على عزلات جديدة من الاستربتوميسس ذات كفاءة عالية في إنتاج المضادات الحيوية لاستخدامها كعوامل للمكافحة الحيوية لاثنين من الفطريات (*Fusarium oxysporium* و*Alternaria solani*) ونوع واحد من البكتريا هو *Streptomyces scabies* وذلك من خلال المعاملة بالمطفرات والدمج البروتوبلاستى. حيث تمت المعاملة بنوعين مختلفين من المطفرات الكيمائية هما النتروزوجواندين و الايثايل ميثان سلفونيت. وقد أستخدم لهذا الغرض نوعيين من الاستربتوميسس هما *S. fradiae* KCC-5-0133 و*S .endus* KCC-5-0213.

وقد أظهرت النتائج ما يلي: (1) تم الحصول على 79 عزلة نتيجة معاملة السلالة *S.fradiae* بتركيزات مختلفة من النتروزوجواندين لمدة ساعة واحدة. أظهرت 17 منهم تفوقا بنسب مختلفة علي السلالة الأصلية في إنتاج المضادات الحيوية. (2) تم الحصول أيضا على83عزلة نتيجة معاملة السلالة *S. endus* بنفس المطفر السابق بتركيزات مختلفة. اظهر 21 منهم تفوقا علي السلالة الأصلية في إنتاج المضادات الحيوية بنسب متباينة. (3) تم انتخاب 100 عزلة عشوائيا بعد معاملة السلالة *S. fradiae* بالمطفر ايثايل ميثان سلفونيت بتركيزات مختلفة لمدة ساعة تفوق منهم 18 عزلة فقط عن السلالة الاصلية فى الإنتاجية. (4) أظهرت 17 عزلة من آلـ 100 عزلة المنتخبة عشوائيا من معاملة السلالة *S. endus* بالايثايل ميثان سلفونيت إنتاجية أعلى للمضادات الحيوية عن السلالة الأصلية. (5) تم الحصول علي 26 مندمجة خلوية نتيجة إجراء تسع تهجينات مختلفة احداهما بين السلالتين الأبويتين والباقي بين العزلات المختلفة الكفاءة والناتجة من معاملة كلا السلالتين الأبويتين بالمطفرين السابقين (عالي/عالي) و (عالي/منخفض) و(منخفض/منخفض). (6) أوضح تقييم هذه المندمجات أن هناك ستة مندمجات فقط اظهروا مقدرة فى إنتاج المضادات الحيوية. وأثبتت ثلاث مندمجات من تلك الست أنها ذات كفاءة عالية عند استخدامهما فى المكافحة الحيوية لنوعى الفطر ونوع البكتريا المستخدمين لهذا الغرض. أوضحت النتائج أيضا أن العشرون مندمجة الباقية قد فقدت قدرتها على إنتاج المضادات الحيوية .