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16S rRNA of *Streptomyces* isolates, mutagenic by EMS and antagonistic tested on *Fusarium*, *Pythium* and *Rhizoctonia* of cucumber plants

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Abstract

Cucumber is one of Egypt's most popular vegetables, although it is sensitive to a number of plant diseases. The current study aimed to develop superior Streptomyces bacteria for improved antagonistic to fungi using mutagenic agents. Two Streptomyces isolates were isolated from Egyptian soil and identified by biochemical methods according to Bergey's Manual and partial 16S rRNA sequencing. These isolates were phenotypically identified as Streptomycetes genera. Mutation experiment by using chemical ethyl methanesulfonate (EMS) mutation methods. Through mutagenesis by (EMS) out of 10 obtained mutants. The mutants of Streptomyces isolates gave height antagonism of cucumber plant fungi Fusarium, Pythium and Rhizoctonia on the plates selected and applied in pots experiments growing cucumber. Streptomyces isolates and their mutants gave high inhibition percentage for the three (the studied) pathogenic fungi compared with the control. In soil infested with *Pythium deberianum* and *Rhizoctonia solani* treatments with mutants 6, 7 and Topsin-M70 fungicide increased the percentage of survival plants by up to 85% compared with the control (40%). While the mutants 5. With *Fusarium oxysporium* 75 %. survival plants. While mutant 4 with *Rhizoctonia solani* and 2 and 9 *Fusarium oxysporium* had the lowest effect on increasing cucumber survival (55%) and mutant 2,8 on *Pythium deberianum* gave 60% compared with other treatments and control. This finding suggests that Streptomyces could be useful in protecting plants such as cucumbers from fungi and reducing environmental contamination with herbicides.

Keywords: *Streptomyces*, mutagenic, antagonistic, biological control, cucumber, 16S rRNA.

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1. Introduction

Cucumber (*Cucumis sativus* L.) is one of the world's most important vegetable crops, and it is grown in Egypt. Cucumber affected by various plant diseases. Damping-off or root-rot diseases are considered among the most important diseases that affect plants and cause high losses in cucumber yield. The primary causes of damping-off or root-rot diseases are *Pythium* spp. such as *Pythium ultimum* (Trow), *Pythium deberianum, Fusarium* spp. such as *Fusariumsolani* (Mart) *Fusarium oxysporium* f.s. *cucumarinum, Rhizoctonia solani* Kuhn, *Thanatephorus cucumeris* (Frank) Donk and *Sclerotinia sclerotiorum* (Lib de Bary) (Haggag, 1997). Constant use of fungicides for diseases control has resulted in several environmental problems such as, long persistence period, pollutive effects (Dubey and Mall, 1972), phytotoxicity (Fawcett ad Spencer, 1970), teratogenicity (Javoraska, 1978) and carcinogenicity (Epstein *et al.*, 1967). These factors highlight the importance of developing new methods for controlling plant diseases (Wilson *et al.*, 1987). Actinomycetes are a phylum of soil-dwelling microorganisms. They are Gram-positive bacteria that produces an assortment of therapeutic compounds (Anandan *et al*., 2016). Streptomycetes produce approximately 7600 chemicals a lot of which are metabolites secondary that are powerful antibiotics making them the most important antibiotic-producing organisms used by pharmaceutical companies. (Ramesh and Mathivanan, 2009). Actinomycetes are also employed as plant growth promoters (by assisting in the production of the plant hormone, biocontrol, biopesticides, antifungal chemicals, biocorrosion as well as a source of agroactive substances (Sharma *et al*., 2014). Actinomycetologists now have a phyletic tree thanks to 16S rDNA sequences, which allows them to investigate the evolution of actinomycetes while also laying the groundwork for identification (Karthik *et al.*, 2010). Reveals that mutation is one of the best strategies for strain formation to improve Actinomycetes' efficiency (Miller, 1977). EMS cause GC to AT transversions. Research have been done in the past employing chemical mutagenic agents to obtain genetic variability in different *Streptomyces* spp. (Lanoot *et al.*, 2005). High incidences of soil-borne diseases during the seedling stage, such as damping-off or root rot, can result in crop losses of 60–90%. *Rhizoctonia solani* Kuhn is a significant soil-borne fungal pathogen that under favorable environmental conditions can cause diseases in a wide variety of plants (Drizou, 2017). It is a facultative parasite that competes fiercely with other organisms soil-borne. In infected soils its survival is primarily due to the formation of sclerotia, which allows it to survive without a host for an extended period of time (Wang *et al*., 2013). The infection and spread of disease are caused by the germination of sclerotia in the presence of a susceptible host. Plants are more susceptible to *R. solani* infection during the seedling stage due to a lack of resistance mechanisms that normally emerge during the adult stage of plant growth. In highly susceptible plants, seedling blight, root rots, and hypocotyl

rots are common symptoms of R. solani infection, especially when planted in favorable environmental conditions (Wang *et al.*, 2015). Biological control of plant pathogens has emerged as a critical strategy for plant disease management. Hence, several successful attempts have been made to control the pathogens by using antagonistic fungi and antagonistic bacteria, which attack the mycelium of causal plant diseases. Different antagonistic fungi or bacteria varied in their action. These variations may be due to the difference in ability of each antagonist to over grow or to produce toxic substance or due to their ability to parasite the hypha of pathogens and to the antibiosis potential (Elad *et al.*, 1982; 1983; Upadhyay and Mukhopadhyay, 1986). The current study was attempted to develop effective Streptomyces genotypes for biological control of fungal pathogenic in cucumber.

2. Materials and methods

Experiments were carried out in the Laboratory of Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt and under the Plastic House of Gemmeiza Agricultural Research Station, Gharbia, Egypt.

2.1 Isolation and identification of causing damping-off and wilt disease of cucumber plants

Samples of diseased cucumber plants showing damping-off typical and root-rot were gathered from various fields. The samples of the diseased cucumber plants with their rhizosphere soil were preserved separately in polyethylene bags and then carry to the laboratory for further studies. The lower stems and roots were washed with tap water to remove any soil particles that had become attached before being cut into small pieces (approx. 2 cm.). The diseased little pieces were surface sterilized in 3% sodium hypochlorite for 3 minutes and rinsed several times in renewed sterilized distilled water and then dried between sterilized filter papers. The sterilized little pieces were placed on potato dextrose agar (PDA) medium and incubated at 27-30^{o}C for 3-5 days before being purified using the hyphal tip technique (Dhingra and Sinclair, 1995). The reisolated fungi were identified according to Gilman (1957), Barnett (1960), Booth (1977) and Singh (1982). The identified fungi were cultured on slants of PDA medium and kept at 4^{o}C for further studies. During the isolation and the identification processes isolates frequency of the different fungal genera were counted. In another trial, 10 gm of the rhizosphere soil were added to 90 ml sterilized distilled water in a 250 ml conical flask and thoroughly shacked for 10 minutes. Dilution series up to (10^{6} cfu /ml) was prepared. Portions of 0.1 ml from the 10^{6} cfu /l dilution were spread on 4 Petri dishes for each sample; containing PDA medium and incubated at 25±2^{o}C for 3 days, then examined microscopically, purified, identified and isolate frequency was calculated. The stock cultures of the isolated pathogenic fungi, namely *Pythium deberianum, Fusarium oxysporium* f.s. *cucumarinum, Rhizoctonia solani* were used for

performance the pathogenicity test; inocula from the previous cultures were grown separately on hulls rice medium. 500 ml glass bottles containing 100 gm hulled rice, 200 g sand, and 100 ml water were autoclaved at 1.5 atm for 20 minutes. Following that, three mycelial discs (6 mm in diameter) taken from the margin of an actively growing colony of a fungal isolate on PDA were added to each bottle of medium. The glass-bottles with hulls rice culture were incubated at 20-28$\pm$2^{o}C according to each fungus for 7 days. Sandy-clay soil was sterilized with a 5% formalin solution before being compacted into 25 cm diameter pots with 4 kg soil per pot. The hull rice culture (w/w) was artificially inoculated in pots one week before sowing at a rate of 3 percent, and the inoculated potted soil was kept moist until sowing. Ten cucumber seeds of the hybrid (Delta star) were sown/pot. Four replicates were used for each treatment. The pots were kept in a greenhouse and were watered as needed. Disease incidence of pre-emergence damping-off was listed 15 days after sowing, while post-emergence damping-off and survived plants were listed 30 days after sowing as follows (Khalifa, 1987):

$$\text{Pre-emergence damping-off (\%)} = \frac{\text{No. of non emerged seeds}}{\text{No. of sown seeds}} \times 100$$

$$\text{Post-emergence damping-off (\%)} = \frac{\text{No. of killed seedlings}}{\text{No. of sown seeds}} \times 100$$

$$\text{Survived plants (\%)} = \frac{\text{No. of survival plants}}{\text{No. of sown seeds}} \times 100$$

2.2 Isolation, purification and identification of Streptomyces isolates

For the isolation of Streptomyces was carried out by pour plate method of dried soil collected from different ecological habitats. soil serial dilution was done as mentioned previously, the highest dilution (10^4 up to 10^6) were inoculated in petri dish one ml/plat and pour 10 ml of sterilized inorganic salts starch medium (ISS), weak agitation after solidification. The inoculated plates incubated at 30^{o}C for 5-6 days, the appear colonies each similarity colonies characterized select one is and more streaked on the same medium to purification. After checked purified Streptomyces isolates as characterization can be performed to study to purify the streak plate method was used. The colony of Streptomyces was identified morphologically as colored, dried, rough, with an irregular/regular margin, and generally convex (Singh and Agrawal, 2003). Using a magnifying glass, colony morphology was observed in terms of color, aerial mycelium, size, colony nature, reverse side color and pigmentation.

2.3 Biochemical characterization of Streptomyces

Various physiological and biochemical tests (starch hydrolysis, casein hydrolysis, Tween 20 hydrolysis, urea-hydrolysis, sugar utilization, citrate utilization test, and catalase test) were performed to characterize wild and mutant Streptomyces isolate (Singh and Agrawal, 2003).

2.4 EMS-mutagenesis

Streptomyces were treated with 0.0, 20, 40, 60, 80 µl of EMS/ml for one hour. Dilutions were spread on spore medium

and incubated at 28ºC for three days. Mutants were colonies with morphologies that differed from the wild type (Adrio and Demain 2006; El-Sherbini and Khattab, 2018).

2.5 Molecular Markers

2.5.1 Total DNA isolation

DNA extraction and purification were performed in accordance with the manufacture's manual of DNeasy blood Tissue Kits (QIAGEN-Germany).

2.5.2 16S rDNA

Sequences of 16S rDNA were used in Streptomyces ologists with a phylogenetic tree that allows them to investigate the evolution of actinomycetes. DNA used as template was amplified by PCR using 16S rRNA 8F (5'-AGTTGATCCTGGCTCAG-3') and reverse primer 1492 R (5'-ACCTTGTTACGACTT-3') with the program include of denaturation at 94°C for 5 minutes and 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 2 min the final extension at 72°C for 5 minutes. PCR products was determined by electrophoresis of 10 µl of the reaction product in a 1% agarose gel by Lane *et al.* (1985) and Isik *et al.* (2014).

2.5.3 Analysis of the 16S rDNA

The 16S rDNA analysis began with analytic DNA (Hapwood *et al.*, 1985) and amplifying the gene coding for 16S rRNA using the enzyme chain reaction (Siva, 2001).

2.6 Biocontrol applied test

The biocontrol was achieved *in vitro* and with positive results were applied in an experimental pots trial.

2.6.1 Evaluation effect of some antagonistic Streptomyces isolates on pathogenic cucumber plant fungi

This experiment was carried out to investigate the antagonistic action of some Streptomyces isolates and their mutant which used against some pathogenic fungi *i.e.*, namely *Pythium deberianum, Fusarium oxysporium* f.s. *cucumarinum* and *Rhizoctonia solani*. Potato dextrose agar (PDA) plates were inoculated with a 6 mm disk of 3–5 days old culture of each pathogen. A disk of 3-5day old culture of the antagonist to be tested fungi was placed at a constant distance opposite to the pathogens disk. While a loopful of 2day old broth culture (10^8 cfu /ml) was streaked at a constant distance opposite to the pathogen disk. All petri-dishes were incubated at 28±1ºC for namely *Pythium deberianum, Fusarium oxysporium* f.s. *cucumarinum, Rhizoctonia solani* **(**Onkar and James, 1995) and observed daily, when the pathogenic fungi nearly covered the surface of the medium in control treatment. Percentage of inhibition was calculated by measuring the pathogenic fungal radial growth according to Topps and Wain (1957) and Ferreira *et al.* (1991) as follows:

$$R\ (\%) = \frac{A - B}{A} \times 100$$

Where: R = Percentage of growth reduction. A = The average of two perpendicular mycelial radial growth of the pathogenic fungus in control. B = The average of two perpendicular mycelial radial growth of the pathogenic fungus in the antagonistic treatment.

2.6.2 Under greenhouse conditions

This experiment was carried out to learn more about the ability of some isolated Streptomyces and their mutant agents to reduce cucumber damping-off disease. The cucumber hybrid Delta star was used the fungicide Topsin-M70 (Dimethyl 1, 2-phenyl bis (amino-carbonothioyl) bis carbamate was used for comparison in addition to the control (treated with pathogenic fungi). Pots of 25 cm diameter were filled with sterilized clay-loamy soil by means of formalin solution 5% and seeded with cucumber hybrid Delta star (5 seeds/pot). Seven days before sowing pots were infested with each individual pathogenic fungus grown on hulls rice medium for 7 days as previously described, at rate 3% of soil weight. Application of the fungal bioagent organisms were performed at the day of sowing, at rate of 3% of the weight of soil and were mixed in the upper 5-cm of the soil surface. Topsin-M70 was used as seed dressing at rate of 3 g/kg seed. Pots of the control were infested only by the pathogenic fungi. While application of the bacterial bioagents were achieved by adding 50 ml of liquid media (10^8 cfu/ml) per pot. Four pots were used for each treatment. Pre-emergence damping-off was recorded after 15 days from sowing. While post-emergence damping-off and survived plants percentages were recorded after 30 days.

2.7 Statistical analysis

A complete block randomized design with four replicates were used in these experiments. Analysis of variance (ANOVA) was performed to calculate L.S.D. value (least significant difference) according to (Snedecor, 1965).

3. Results and Discussion

3.1 Identification and frequency of the isolated pathogenic fungi causing damping-off cucumber

The results in Table (1) revealed that the major pathogenic fungi which cause damping-ff on cucumber plants according to their frequency were *Fusarium oxysporium* f.s. *cucumarinum, Rhizoctonia solani* while and *Pythium deberianum*. *Fusarium solani* and *Sclerotinia sclerotiorum* were less importance in inducing the disease incidence. Results presented in Table (1) indicate that main five fungal pathogenic species belong to four genera were isolated from infected cucumber plants and their rhizosphere soil. Percentages of their frequency revealed that *Fusarium oxysporium* f.s. *cucumirina, Rhizoctonia solani* followed by *Pythium deberianum* were the most prevalent fungi giving the highest frequencies, hence their frequency percentages from the infected plants were 27.52, 25.11 and 21.87%, respectively and 27.33, 23.33 and 20.93%, respectively from their rhizosphere soil.

On the other side, frequency percentage of *Fusarium solani* and *S. sclerotiorum* were 12.64 and 9.84% when isolated from the diseased plants and 13.36 and 9.78 % from their rhizosphere soil, respectively. Some other unidentified fungi were recorded which their frequencies were 3.02 and 5.32% from the diseased plants and their rhizosphere soil, respectively.

3.2 Pathogenicity test

The pathogenicity test showed that the four isolates of each fungus, *i.e*. that *Fusarium oxysporium* f.s. *cucumirina*, *Rhizoctonia solani* and *Pythium deberianum* were virulent as they caused damping-off on cucumber seedlings (*Cucumis sativus* L. cv. Delta star) by rates ranged from 17.5 to 35.0 %, 20 to 32.5% and 22.5 to 30.00 of pre-and post-emergence damping-off, respectively (Table 2). However, there are significant differences among the four isolates of each fungus in virulence. The most virulent isolates were No. 2, 3 and 1 of *Fusarium oxysporium* f.s. *cucumirina*, *Pythium deberianum* and *Rhizoctonia solani* respectively, since the percentage of survived plants were 32.5, 37 and 35%, respectively, compared with the control treatments 100%.

Table (1): Isolation frequency of soil-borne pathogens from infected cucumber plants and rhizosphere soil.

Isolated fungi	Isolation frequency (%)		Mean (%)
	From diseased plants	From rhizosphere	
Fusarium oxysporium f. sp. *cucumirina*	27.52	27.33	27.42
Pythium deberyanum	21.87	20.93	21.40
Rhizoctonia solani	25.11	23.33	24.22
Fusarium solani	12.64	13.36	13.00
Sclerotinia Sclerotiorum	9.84	9.73	9.78
Others	3.02	5.32	4.17
L.S.D. at 5%	5.77	5.42	5.59
L.S.D. at 1 %	7.55	7.32	7.43

Table (2): Pathogenicity test of the most frequent four isolates from each of *F.oxysporium*, *P.* deberyanum and *R. solani* fungi on cucumber hybrid Delta star sown in pots under greenhouse conditions.

Isolates no.	*F. oxysporium*			*P. deberyanum*			*R. solani*		
	Pre emergence (%)	Post emergence (%)	Survived plants (%)	Pre emergence (%)	Post emergence (%)	Survived plants (%)	Pre emergence (%)	Post emergence (%)	Survived plants (%)
1	22.50	20.00	57.50	20.00	27.50	52.50	30.00	25.50	35.00
2	35.00	27.50	32.50	22.50	22.50	50.00	25.00	22.50	52.50
3	17.50	20.00	62.50	32.50	30.50	37.00	22. 50	22.50	55.00
4	27.50	22.50	50.00	30.00	32.50	37.50	27. 50	25.00	47.50
Control	0.00	0.00	100.00	0.00	0.00	100.0٠	0.00	0.00	100.00
L.S.D. at 5%	7.54	6.30	9.68	6.96	7.04	8.71	8.17	6.38	9.66
L.S.D. at 1%	8.63	7.89	9.78	8.29	8.34	9.28	8.98	7.94	9.77

On the other side, the isolates No. 3 of *Fusarium oxysporium*, No. 1 of *Pythium deberianum* and No. 3 of *Rhizoctonia solani* were the least pathogenic tested isolates, since the percentage of survived plants were 62.5, 52.5 and 55 %, respectively. Henz and Lima (1998) and Trusevich *et al.* (2002) who reported that *Fusarium* spp., *P. deparyanum*, *P. ultimum Rhizoctonia solani* and *S. sclerotiorum* were widely spread in cucumber as causal organisms of damping-off and root-rot.

3.3 Identification of actinobactria by 16S rRNA

Bacterial identification based on phenotypic characteristics is general traditionally less accurate than genotypic identification. For species separation and identification of Streptomycetes DNA based molecular approaches have been applied. (Isik *et al.*, 2014). The nucleic acids recovered from created bacterial colonies which amplify the 16S rRNA region and were all produced by expected size ~1550 bp given a main pair name as shown in the Figure (1).

3.4 Sequence of 16S rRNA and PCR

The 16S rRNA sequence is a long of ~1550 bp and contains both changeable and conserved loci. With enough interspecific variations in the 16S rRNA sequence is large enough to discriminate Attallah *et al*. (2014)**.** Within the science of bacteria and actinomycetes, the significance of phyletic research supported by 16S rDNA sequences is growing (Yokota, 1997). The sequence is the variable area between these sections (Chen *et al.*, 1989). Regardless of its accuracy 16S rRNA sequence analysis is not widely used outside of big and reference laboratories due to technical and financial constraints (Figures 1 and 2) (Clarridge, 2004).

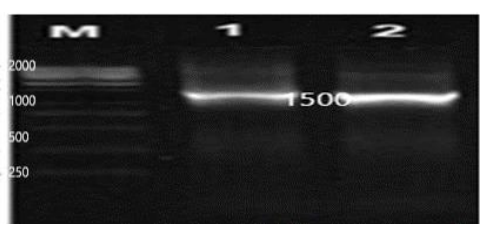

Figure (1): The two Streptomyces isolates amplified by universal primers were the full length 16S rRNA (~1500 bp) gene. Amplicon solved electrophoretically in 1X TBE buffer using a 1.2% agarose gel Lane1: DNA ladder, Lane 1-3: full-length amplified 16S rRNA product.

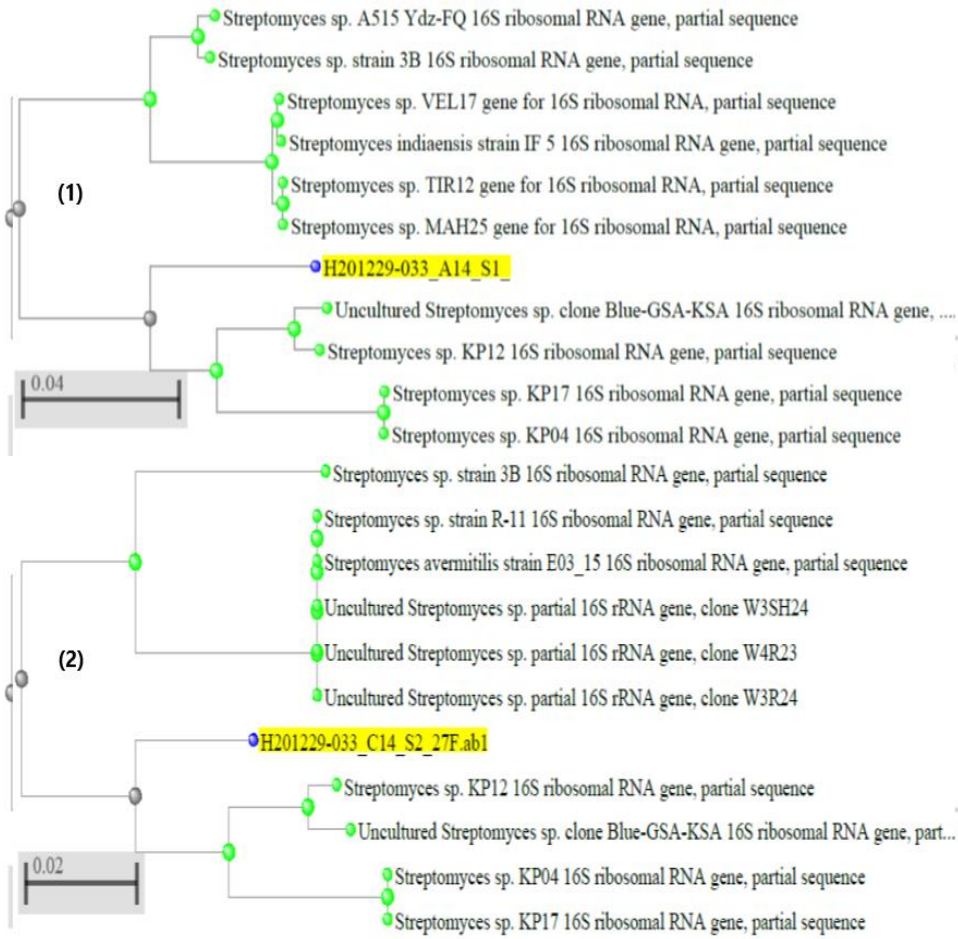

Figure (2): The relation between the two isolates and the remaining species belonged to the genus Streptomyces, based on the partial 16S rDNA sequences. Phylogenetic tree has been built using the next method CLUSTAL-X. 1=*Streptomycetes* sp. 2= *Streptomyces avermitilis* strain.

The 16S rRNA sequences may be compared to discriminate between organizations on the level genus throughout all major bacterial phyla in addition to multi-leveled strains including what is now known as the species and subspecies level. In this investigation, the 16S rRNA sequences of two Streptomyces isolates from Egyptian soil were determined. A phylogenetic tree depend on 16S rRNA sequences was built using 50 GenBank 16S rRNA Streptomyces partial sequences.

3.5 Phylogenetic analysis and similarity of 16S rRNA sequence gene

Using BLAST, we discovered that the two strains belonged to the Streptomyces

species. The BLAST similarity values varied between 98 and 95 percent. The species identification of the two strains was established by a molecular analysis of the 16S rRNA. 1st query sequence was substantially like the strain of *Streptomyces* sp. VEL17. Strain *Streptomyces avermitilis* E03 15 is linked to the remaining strains. These results agree with Rintala *et al*. (2001) who identified Streptomycetes by 16 S rRNA (Baskaran *et al*., 2017).

3.6 Mutation induction response of Actinobacteria

As previously stated in the adopted materials and techniques the two Streptomyces were exposed to EMS mutagen. After EMS mutagenesis the survival percentages significantly dropped with increasing doses treatment as shown in Table (3). The exposure concentration (20 µl/ml) had the highest survival percentage followed by those derived from spore suspensions subjected to concentrations of 40, 60, and 80 µl/ml EMS. In both Streptomyces10 single random colonies are isolated from the 20,40,60 and 80 µl/ml treatments to determine their antibacterial efficacy on fungus under study. The results are similar with Khattab and El-Bondkly (2006) and El-Sherbini and Khattab (2018) said that UV and EMS are two of the most used mutagens for bacterial strain improvement producing a variety of point mutations in Streptomyces bacterial DNA and El-Fadly *et al*., 2009 in Streptomyces bacteria and in *Bacillus* spp. (Sayed *et al*., 2016).

3.7 Characterization of Streptomyces and their mutants

Table (4) show two strains of Streptomyces isolates and their 10 mutants were able to hydrolyses starch, tween 20 and casein utilize fructose and lactose. All of them tested positive for urease, citrate utilize and catalase. In colony color, colony texture, or both, all mutants were discovered to be distinct from their wild counterparts (Table 4). These results agree with Atalan *et al*. (2000).

Table (3): Effect of EMS on viability of *Streptomyces* and its mutants for one hour.

Concentration of EMS (µl/ml)	*Streptomyces sp*.		No. of mutant	*Streptomyces avermitilis*		No. of mutant
	No. of cell survived/ml (10^5)	Survival (%)		No. of cell survived/ml (10^5)	Survival (%)	
0.0 (control)	320	100	0	350	100	0
20	160	50.00	1	204	58.28	1
40	111	34.68	1	192	54.85	1
60	45	14.06	2	102	29.14	2
80	10	3.12	1	22	6.28	1
Total No. of mutants			5			5

Streptomyces isolated from environmental Northumberland, UK. Representative isolates were found to have properties consistent with their

classification in the genus Streptomyces and were recovered in three taxa using different phenotypic criteria, namely morphological and pigmentation properties. Actinomycetes can be isolated using a variety of methods based on different sources and media (Sharma *et al*., 2014).

Table (4): Biochemical characterization reaction of two moderately sensitive Streptomyces strains and their mutations by EMS.

Strains	Colony color	hydrolyses starch	Tween 20	Casein utilize	Fructose	Lactose	hydrolyses urea	Citrate utilize	catalase
Streptomyces sp.	Gray	+++	+++	+++	+++	+++	+++	++	++
Mutant 1	Gray	+++	+++	+++	+++	+++	+++	++	++
Mutant 2	Gray White	+++	+++	+++	+++	+++	+++	++	++
Mutant 3	Gray White	+++	+++	+++	+++	+++	+++	++	++
Mutant 4	Gray White	++	++	++	++	++	++	+	+
Mutant 5	Gray White	++	++	++	++	++	+++	++	+
Streptomyces avermitilis	yellow	+++	+++	+++	+++	+++	+++	++	+
Mutant 6	yellow	+++	+++	+++	+++	+++	+++	++	++
Mutant 7	yellow	+++	+++	+++	+++	+++	+++	++	++
Mutant 8	Bright yellow	+++	+++	+++	+++	+++	++	++	++
Mutant 9	Bright yellow	++	++	++	++	++	++	++	++
Mutant 10	Bright yellow	++	++	++	++	++	++	++	++

3.8 Effect of certain Streptomyces strains and their mutants antagonists on the causal fungal pathogen fungi and on the cucumber damping-off incidence

3.8.1 Effect on linear growth of the pathogenic fungi

Actinomycetes are common filamentous soil microorganisms that play an important role in maintaining a healthy biological balance in the soil, owing to their ability to produce antibiotics (Sujatha, 2018). Data showed in Table (5) and Figures (3, 4 and 5) show that there were significant differences in inhibition, pathogenic fungi grow in a straight line because of using the different antagonistic Streptomyces isolates and their mutant comparing to the control treatments. Data revealed that inhibition percentages of *F. oxysporium* mutant 6 were the most effective hence it gave 15.3 where mutant 10 of the lesse effective (22.7).

Table (5): Antagonistic effect of Streptomyces isolates and their mutants onlinear growth of Fusarium, Pythium and Rhizocotonia by dualplate method.

Isolate no.	Tested fungi / Antagorganism	*F. oxysporium* (zone/mm)			Mean (mm)	*P. deberyanum* (zone/mm)			Mean (mm)	*R. solani* (zone/mm)			Mean (mm)
1	*Streptomyces* sp.	18	15	21	18.0	23	24	21	22.7	17	22	21	20.0
2	Mutant 1	22	17	23	20.7	22	25	23	23.3	20	21	20	20.3
3	Mutant 2	23	23	21	22.3	23	19	24	22.0	23	17	18	19.3
4	Mutant 3	18	24	22	21.3	19	21	21	20.3	17	16	22	18.3
5	Mutant 4	19	23	21	21.0	18	22	22	20.7	19	19	23	20.3
6	Mutant 5	16	17	16	16.3	17	18	19	18.0	16	19	18	17.7
7	*Streptomyces avermitilis*	22	21	18	20.3	23	24	24	23.7	24	23	23	23.3
8	Mutant 6	15	15	16	15.3	14	13	13	13.3	18	18	22	19.3
9	Mutant 7	22	23	16	20.3	15	22	22	19.7	22	17	15	18.0
10	Mutant 8	23	23	22	22.7	17	23	17	19.0	17	19	21	19.0
11	Mutant 9	24	12	23	19.7	18	14	13	15.0	21	13	22	18.7
12	Mutant 10	21	16	16	17.7	19	17	22	19.3	15	12	22	16.3
L.S.D.	at 5%	3.3	4.0	3.3	3.0	3.4	3.9	3.8	3.5	3.3	3.5	3.0	2.6
	at 1 %	5.7	6.3	5.7	5.4	5.8	6.2	6.1	5.8	5.7	5.9	5.5	5.0

The percentages of *P. deparianum*, mutant 6 were the most effective hence it gave 13.3 where Streptomyces. *avermitili*s is the less effective one gave 23.7. *Rhizoctonia solani* mutant 10 were the most effective hence it gave 17,7 where *Streptomyces avermitilis* is the less effective (23.3). The obtained results of biological control assay were in parallel with those reported by Crawford *et al.* (1993) Actinomycete strains isolated from four rhizosphere-associated isolates were screened for *in vitro* antagonism to *Pythium ultimum*. Five isolates were very strong fungus antagonists and ten others were soft antagonistic. These results agree with Baskaran *et al*. (2017) *Streptomyces* extracts from ten isolates were found to be bioactive against two bacteria. Actinobacteria found in sponges may also protect the sponge from bacteria, fungi, viruses, and other microflora by secreting secondary metabolites on the sponge's surface and inside. On the other hand, the obtained results showed good harmony with El-Fadly *et al*. (2009) since they carried out *Streptomyces* mutagenic treated isolates have antagonistic effect against *A. solani*, *F. oxysporium* and *Streptomyces scabies* pathogenic organisms. The obtained results of biological control assay were in parallel with those reported by Sujatha (2018) in *Streptomyces violatus* isolated from rhizosphere of cotton has great antifungal activity and it was extra effective against *Macrophomena phaseolina* when compared with other test fungi *R. solani*, *F. moniliformae*, *A. alternata* and *A. niger*.

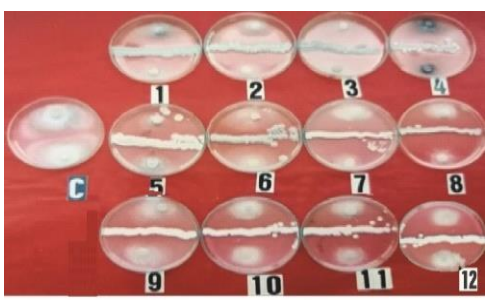

Figure (3): Antagonistic effect of Streptomyces strains on Fusarium *in vitro*.

Figure (4): Antagonistic effect Streptomyces strains on Pythium *in vitro*.

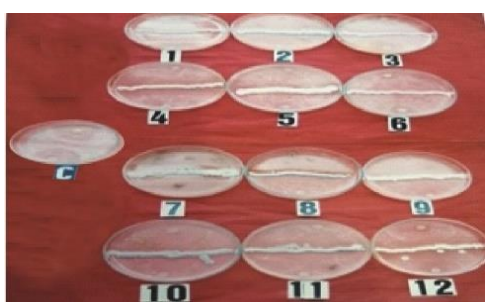

Figure (5): Antagonistic effect of Streptomyces strains on Rhizocotonia *in vitro*.

3.8.2 Effect of the antagonistic Streptomyces isolates on causing damping-off diseases incidence of cucumber sown in pots

Data in Table (6) revealed that treatment with Topsin-M70 fungicide and Streptomyces sp mutant 5 were the most effective in controlling *F. oxysporium* damping-off disease; hence it gave the highest percentages of survival plants 85 % and 75%. While treatment with mutant no. (9) of Streptomyces *avermitili*s and no. (2) of *Streptomyces* sp. had the least effective antagonistic and produced 55.0 % survived plants, but it still significantly more than the control (50.0%) all the other treatment fell in between. However, when soil was infested with *P. deparyanum* the data clearly indicated that Topsin-M70 and *Streptomces* mutant (7) of Streptomyces. *avermitili*s were the most effective in increasing the survival plants 85% while mutant 2 of Streptomyces. *avermitili*s where the least affective treatment gave 60 % survival plants compared with the untreated control (40% survival plants).

Table (6): Effect of certain Streptomyces strains and their mutants antagonists in percentage of pre, post-emergence damping off and survived plants of cucumber plants grown in soil infested with *F. oxysporium, P.* deberyanum or *R. solani* in pots.

Pathogenic fungi / Antagonistic	*F. oxysporium*			*P. deberyanum*			*R. solani*		
	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survived plants (%)	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survived plants (%)	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survived plants (%)
Streptomyces sp.	15.00	20.00	65.00	20.00	10.00	70.00	15.00	15.00	70.00
Mutant 1	15.00	15.00	70.00	20.00	15.00	65.00	15.00	15.00	70.00
Mutant2	20.00	25.00	55.00	15.00	25.00	60.00	10.00	10.00	80.00
Mutant3	15.00	25.00	60.00	15.00	15.00	70.00	20.00	20.00	60.00
Mutant4	15.00	20.00	65.00	20.00	15.00	65.00	20.00	20.00	55.00
Mutant5	10.00	15.00	75.00	10.00	15.00	75.00	15.00	15.00	80.00
*S. avermitili*s	15.00	20.00	65.00	20.00	20.00	60.00	20.00	20.00	65.00
Mutant6	15.00	15.00	70.00	10.00	5.00	85.00	20.00	20.00	60.00
Mutant7	10.00	25.00	65.00	15.00	25.00	60.00	10.00	10.00	85.00
Mutant8	10.00	20.00	60.00	10.00	10.00	80.00	15.00	15.00	65.00
Mutant 9	20.00	25.00	55.00	10.00	10.00	80.00	20.00	20.00	60.00
Mutant 10	15.00	15.00	70.00	15.00	20.00	65.00	15.00	15.00	65.00
Topsin-M70	5.00	10.00	85.00	10.00	5.00	85.00	5.00	10.00	85.00
Control	20.00	30.00	50.00	35.00	25.00	40.00	25.00	35.00	40.00
L.S.D. at 5%	4.0	4.6	5.8	5.1	5.1	6.8	4.5	4.9	6.9
L.S.D. at 1 %	6.3	6.7	7.6	7.1	7.1	8.2	6.6	7.0	8.2

In soil infested with *R. solani* treatments with *Streptomces avermitili*s. Mutants (7) and Topsin-M70 fungicide increased the percentage of survival plants (85 %), while mutant 4 of *Streptomyces* sp. gave the lowest effect in increasing survive plants 55% compared with the control (40%). These results agree with Baskaran *et al*. (2017). *Streptomyces* extracts from ten isolates were found to be bioactive against two bacteria. Actinobacteria may also protect the sponge from pathogenic bacteria, fungi, viruses and other microflora by secreting secondary metabolites on the surface and inside the sponge. Actinomycetes are also used as biocontrol agents, biopesticides, antifungal, biocorrosion, and as a source of compounds agroactive (Sharma *et al*., 2014). Actinomyces play an important

role in the production of antimicrobial agents as well as other industrially important substances such as enzymes. The potential of this could open up exciting new avenues in biotechnology and biomedical research. Actinomycetes appear to be both quantitatively and qualitatively important in the rhizosphere, according to evidence (Barakate *et al.* 2002; Crawford *et al.*, 1993; Doumbou *et al*., 2001, Miller *et al.*, 1990). *Streptomyces griseoviridis strain* K61 is used to treat European flowers, potted plants, greenhouse cucumbers, and a variety of alternative vegetables by root dipping or growth nutrient treatment (Mohammadi and Lahdenpera ,1992). Mycostop^{TM} is a biofungicide containing *S. griseoviridis* as the active component. This output is available in the United States and Europe in some cases, many properties associated with actinomycetes may justify their ability to act as biocontrol tools (Tahvonen, 1982). These characteristics include the ability to colonize plant surfaces, antibiosis against plant pathogens, extracellular protein synthesis, and phytotoxin degradation. Crawford *et al.* (1993) obtained similar results because isolated actinomycete strains from the rhizosphere that showed antifungal activity in the *in vitro* assay were also tested for their effects on the germination and short-term growth of lettuce plants in glasshouse pot.

4. Conclusion

This research suggests that Streptomyces may be beneficial in plant protection like cucumber from destroy off fungi. The efficiency of Streptomyces mutagenic treatment isolates for antagonistic effect fungal pathogenic organisms for cucumber plants *in vivo* and in soil was improved by EMS. The restriction on the use of fungicides and bacteriocides, this growth inhibition phenomena will result in less contamination in the environment.

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