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Role of Some Soil Bacteria and Actinomycetes in Controlling Cucumber Root-Rot Disease

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TWENTY FIVE isolates of N_2-fixing bacteria and eleven actinomycetes were chosen from 117 microorganisms isolated from desert soils and different plant roots to study their effect on *Rhizoctonia solani*, the causative of the root-rot disease. The data revealed that *Azotobacter* R_f and *Azospirillum* K_c were the most active for nitrogen fixation (210 and 133 ppm), root colonization (61.3% and 65%) and antagonistic effect on the pathogenic fungus *R. solani* (15 and 17 mm). The two strains were identified as *Azotobacter chroococcum* and *Azospirillium lipoferum*.

The actinomycetes isolate, *Streptomyces* N_{cu} was the most active strain for root colonization ability (41.4%) and antagonistic effect against *R. solani* (22 mm). This strain was completely identified as *Streptomyces lydicus*.

Keywords: *Azotobacter chroococcum, Azospirillum lipoferum, Streptomyces lydicus, Rhizoctonia solani*, Root rot.

The use of chemical fungicides is undesirable mean for disease control as they are expensive, cause environmental pollution and may induce pathogen resistance (Larson, 1987). Therefore, the development of alternative, more efficient and safe biological methods for plant disease control is an important approach of agricultural biotechnology (Benhamou and Chet, 1993 and Cook, 1993).

The utilization of some microorganisms as biological agents is considered to be of extreme importance due to their positive effects on plant growth (Reddy *et al.*, 1991; Gowily *et al.*, 1993 and El-Shanshoury, 1994).

The role of N_2-fixing bacteria in growth promotion and biocontrol of the causal organism of the cucumber damping off disease in Egypt was studied by Hassouna *et al.* (1998), who reported that a mixture of the used bacteria reduced the growth of the pathogen.

Actinomycetes also play an important role in controlling plant diseases as a biocontrol agents. Tahvonen & Lahdenpeara (1988), Trego & Ruben (1997) and Song *et al.* (1998) showed that some species of *Streptomyces* have antagonistic effect against some pathogenic fungi which causes diseases of many plants.

Microorgansisms that colonize roots are also ideal as biocontrol agents of soil borne disease (Weller, 1988; Nemee *et al.*, 1996 and Schloter & Hartmann, 1998).

The aim of this work was to study the ability of N_2 fixing bacteria and some actinomycetes to control the causal organism of the root-rot disease of cucumber plant and to colonize its roots.

Material and Methods

Isolation of the causal organism of cucumber root-rot disease

The pathogenic fungus was isolated from rotted cucumber roots collected from Noubaria city. The infected roots were washed with tap water, surface sterilized by immersion in 1% sodium hypochloride for 2-3 min, then washed with a series of sterile water and dried between two pieces of filter paper. The infected roots were then cut into pieces under aseptic conditions and plated onto petri-plates containting Czapek's agar medium supplemented with streptomycin to eliminate bacterial growth and incubated at 28°C for 3 days. Hyphae that grew from the root cuts were purified and identified according to Parameter and Whitney (1970).

Isolation and purification of Azotobacter, Azospirillum and Actinomycetes isolates

Fifteen *Azotobacters,* 10 *Azospirilla* and 11 actinomycetes isolates were choosen from 117 isolates obtained from sandy soil samples-cultivated by different crops. They were grown on nitrogen deficient Ashby's medium (Abd El Malek and Ishac, 1968), for *Azotobacter,* Dobereiner's semi-solid malate medium (Dobereiner, 1978) for *Azospirillum* and starch-nitrate agar, (Waksman and Lechevalier, 1962) for actinomycete for 7 days and then kept at 4-5°C for each until used.

Activity of Azotobacters and Azospirilla for N_2-fixation

The purified 25 *Azotobacters* and *Azospirilla* isolates were tested for their N_2-fixing capacity by the micro kjeldahl method as described by Jackson (1958).

Antagonistic effect of N_2-fixing bacteria and actinomycetes against R-solani

The 36 purified isolates of N_2- fixing bacteria and actinomycetes were tested for their antagonistic activities against *R.solani* according to the method described by Waksman (1959) and Hasegawa *et al.* (1990).

Root colonization capacity for the isolates

The most active nitrogen fixing and antagonistic isolates of *Azotobacter, Azospirillum* (2 isolates for each) together with two most active antagonistic actinomycetes were tested for their capacity to colonize the roots of cucumber plant using the plate test method according to Kortemma *et al.* (1994). The root colonization was calculated as the percentage of colonized root length to the total root length.

Selection and identification of the most efficient Azotobacters, Azospirilla and actinomycetes isolates

The most active isolates of *Azotobacters, Azospirilla* (one strain of each) in N_2-fixation, antagonistic activity and root colonization capacity were identified according to their morphological and physiological characteristics using the methods described in Bergey's Manual of determinative Bacteriology (1974). Media as well as methods used in this identification were described by Shirling and Gottlieb (1966).

The most active isolate of actinomycetes in antagonistic activity as well as root-colonization ability was completely identified according to the Bergey's Manual (1984).

Results and Discussion

Isolation and identification of the causal organism

Samples of rotted cucumber roots were collected from fields in Noubaria city and used for isolating the pathogenic fungus. It was identified as *Rhizoctonia solani* (Fig. 1). In this respect, Ceresini & Souza (1997) and Otten & Gilligan (1998) reported that *R.solani* is one of the major pathogen involved in the root-rot and damping off diseases of many plants.

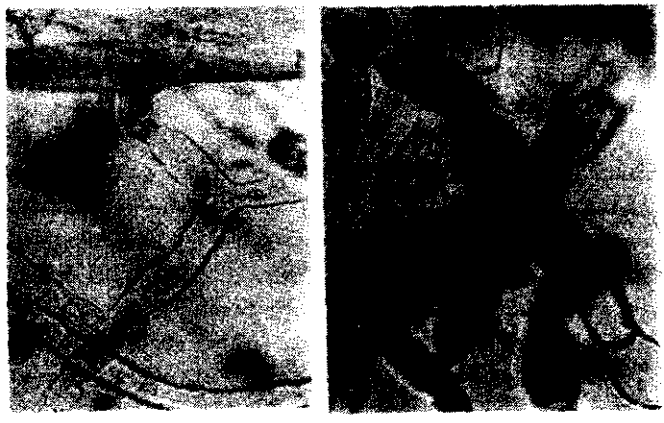

Fig. 1. Vegetative cells of Rhizoctonia solani (x1000).

Activity of Azotobacter, and Azospirilla for N_2-fixation

The data presented in Table 1 show that isolate R_f of *Azotobacters* was the most active in fixing nitrogen being (210 ppm) followed by isolates k_c and k_o being (202 ppm). On the other hand, less active isolate was R_u being (20 ppm).

With respect to *Azospirilla* isolates, the results revealed that, the most active isolates in fixing nitrogen were k_c and k_o being (133 ppm) followed by M_w and M_{co} being (115 ppm) while the lowest *Azospirillum* isolate R_f being (91 ppm).

In this respect, Burris *et al.* (1978) showed that *Azospirillum lipoferum* is an interesting bacterium capable of fixing atmospheric nitrogen in free state or in association with the roots of plants. The fixed nitrogen by *Azotobacter* made available to plants and production of antifungal antibiotics (Shende *et al.*, 1979).

Antagonistic effect of N_2-fixing bacteria and actinomycetes isolates against R-solani

Data presented in Table 1 and Fig. 2 show inhibition zones of different diameters for N_2-fixing bacteria and actinomycetes isolates against *R. solani.* For *Azotobacter* isolates, isolate R_f was the most active antagonistic effect to *R.solani* being (15 mm), followed by k_c and R_{co} isolates (10 mm) for each. Five isolates were with less activity (3-8 mm) while the remaining 7 isolates failed to reduce the growth of the pathogen.

With respect to *Azospirillum* isolates, the most active antagonistic effect to *R. solani* was isolate k_c with inhibition zone of (17 mm) in diameter, followed by isolate k_o (14 mm). The rest proved to be weak against *R.solani* growth whereas they gave inhbition zones ranging from 0 to 9 mm in diameter. In this respect, Cho and Chung (1998) reported that *Azospirillum* sp and *Pseudomonas* sp inhibited the growth of *Fusarium* sp, *Pythium* sp and *Rhizoctonia solani* infecting cucumber and tomato plants.

For actinomycetes, the two isolates N_{cul} and F_{cu} proved to be the most active with inhibition zones diameter of 22 and 20 mm, respectively. Three isolates were less active as they gave inhibition zones not exceeding 7 mm in diameter. Six isolates were non antagonist to the pathogen (Table 1 and Fig. 2). These findings were previously interpreted by Schmiedeknecht (1993); Song *et al.* (1998) and Chamberlain & Crawford (1999) reported that *Streptomyces sp* produced antibiotics which controlled root – rot diseases caused by *R. solani.* Trego and Ruben (1997) found that some *Streptomyces* species reduce the growth of plant pathogenic fungi.

TABLE 1. N_2-fixing activities of *Azotobacter* and *Azospirilla* isolates and antagonistic activities of *Azotobacters*, *Azospirilla* and *Streptomyces* against *Rhizoctonia solani*.

Isolate	*Azotobacter*		*Azospirilla*		*Streptomyces*	Crop
	Total N (ppm)	Inhibition Zone (mm)	Total N (ppm)	Inhibition zone (mm)	Inhibition Zone (mm)	
R_f	210	15	91	5	0	Foeniculum
R_w	100	3	-	-	0	Wheat
R_{co}	90	10	-	-	0	Corn
R_u	20	0	-	-	0	Uncultivated
R_b	115	0	93	0	-	Barley
K_c	202	10	133	17	5	Cuminum
K_o	202	0	133	14	0	Ocimum
N_{cu1}	150	5	100	9	22	Cucumber
N_{cu2}	110	5	95	7	5	Cucumber
N_w	40	0	110	3	-	Wheat
N_{co}	190	0	-	-	-	Corn
F_{cu}	200	0	101	0	20	Cucumber
F_b	100	0	-	-	-	Barley
M_w	45	8	115	0	0	Wheat
M_{co}	95	7	115	4	7	Corn

R_f, R_w R_{co} R_u R_b : 10th of Ramadan soil K_c, K_o : El Khatatba soil. N_{cu1}, N_{cu2}, N_w, N_{co} : Noubaria soil. F_{cu}, F_b : Fayoum soil. M_w, M_{co} : Maryout soil.

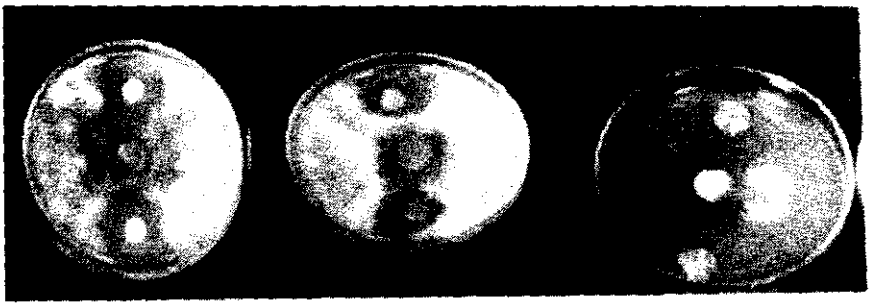

Fig. 2. Antagonistic activity of the most active isolates against R. solani.
A : *Azospirillum* K_c. B : *Azotobacter isolate* (R_f)
C : *Streptomyces isolate* N_{cul}.

The most active isolates *Azotobacters* (R_f & K_c),*Azospirillum* (K_c & K_o) and actinomycetes (N_{cul} & F_{cu}) were tested for their ability to colonize cucumber roots. The results represented in Table 2 reveal that. The four isolates of nitrogen fixing bacteria *Azotobacters* R_f & K_c and *Azospirilla* R_{kc} & K_{ko} were active in colonizing cucumber roots ranged descendingly from *Azospirillum* K_c (65%) to *Azotobacter* K_c (51.3%). With regard to actinomycetes isolates (N_{cul} & F_{cu}), the isolate N_{cul} was the most active in colonizing cucumber roots (41.4%) while the isolate F_{cu} was the least active one (26.6%) compared to all tested microbes. In this respect, Gras and Fernendez (1993) found that inoculation with *Azospirillum lipoferum* increase yields of cucumber by 57-114%; Sanhita *et al.*(1995) reported that inoculation with *Azospirillum* sp and *Azotobacter chroococcum* increase dry weight, root and shoot length, Weller (1988) and Nemec *et al.* (1996) reported that the microorganism that colonizes the roots is ideal for use as a biocontrol agent against soil borne disease.

TABLE 2. Effect of inoculation with biofertilizer agents on root colonization of cucumber seedlings.

Microbial isolates	Root length (cm)	Root colonization length (cm)	Root colonization (%)
Azotobacter R_f	10.1	6.2	61.3
Azotobacter k_c	7.4	3.8	51.3
Azospirillum K_c	10	6.5	65.0
Azospirillum K_o	9.5	5.3	55.7
Streptomyces N_{cul}	8.2	3.4	41.4
Streptomyces F_{cu}	7.5	2.0	26.6

Control : Uninoculated root length = 7.25 cm.

Selection and identification of the most active isolates

Data presented in Tables 3 & 4 and Fig. 3 & 4 show the characteristics of the most active bacteria (R_f & K_c) in fixing nitrogen, inhibiting the growth of pathogenic fungus *R.solani* and root colonization ability. According to Bergey's Manual (1984), they were identified as *Azotobactar chroococcum* and *Azospirillum lipoferum*, respectively.

With respect to the most active actinomycetes isolate N_{cul} in inhibiting *R-solani* and root colonization, it was identified according to their biological and chemical characteristics. The data recorded in Table 5 show that the isolate gave a good aerial mycelium at 30°C after 14 days of incubation period on all the used media. It produced non-fragmenting substrate mycelium with well branching aerial hyphae carrying long spiral chains of spores. Spores are cylindrical to oval in shape with smooth surface, (Figs. 5 & 6).

TABLE 3. Identification of *Azotobacter* isolate (R_f).

Biochemical reaction	Shape
- Cell morphology	Ovoid shaped in pairs
- Sucrose as sole carbon source	+
- Manitol as sole carbon source	+
- Benzoate as sole carbon source	+
- Motility	-
- Reduction of NO_3	+
- Catalase production	+
- Starch hydrolysis	+
- Production of non diffusible pigment	Brown pigment

TABLE 4. Identification of *Azospirillum* isolate (K_c).

Biochemical reaction	Shape
- Cell morphology	Slightly curved and straight rods
- Motility	+
Sucrose as sole carbon source	-
Glucose as sole carbon source	+
-α - Ketoglutaric acid as sole c-source	+
- Reduction of nitrate	+
- Catalase production	+
- Acidification of glucose	+
- Biotin requirement	+

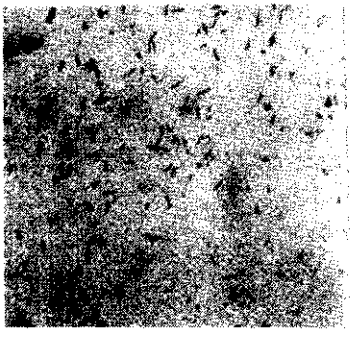

Fig. 3. Vegetative cells of *Azospirillum lipoferum* (x 1000).

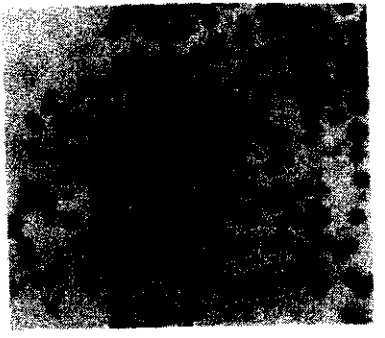

Fig. 4. Vegetative cells of *Azotobacter chroococcum* (x 1000).

TABLE 5. Cultural, Morphological and Physiological Characteristics of *Streptomyces* isolate N_{cul}.

Character	
Colour of aerial mycelium	Grey series
Colour of substrate mycelium	Yellowish brown
Diffusible pigments	Not produced
Spore chains	Spiral
Spore surface*	Smooth
Melanoid pigment	Not produced
Growth on Czapek's medium	Moderate
D-Glucose	+
D-xylose	+
L-Arabinose	+
L-Rhamnose.	-
D-Fructose	+
Raffinose	+
D-Mannitol.	+
Inositol	+
Sucrose	+
Utilization of sole nitrogen source	+
Antagonistic activity	Antifungal, slight anti bacterial
Sensetivity to streptomycin	Inhibited
NaCl tolerance	$\geq$ 10%
Remark.	Hygroscopic

* Examined by electron microscope

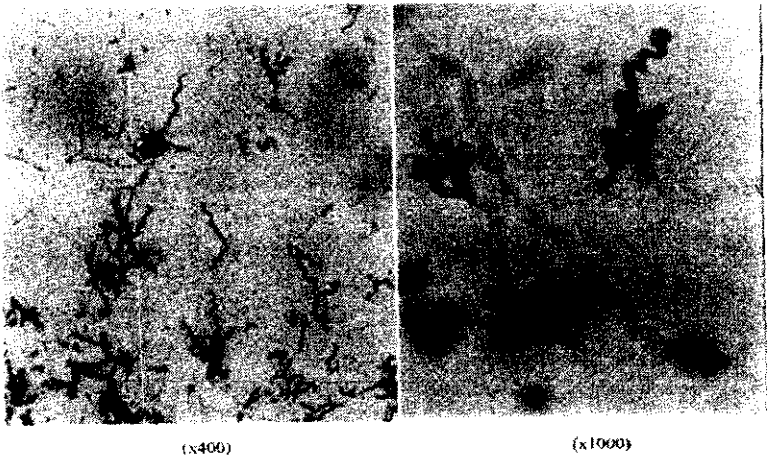

Fig. 5. Micromorphology of spore chains of *Streptomyces lydicus*.

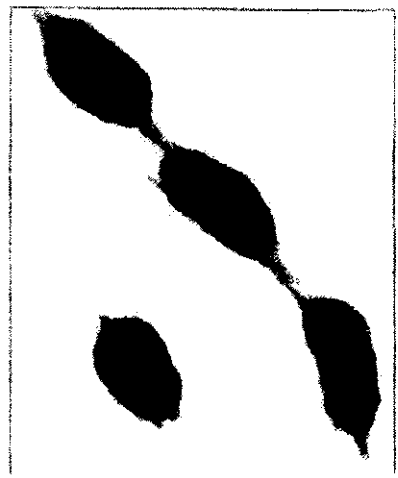

Fig. 6. Electron micrograph of spore morphology of *Streptomyces lydicus*.

Cultural properties of the isolate showed that the colour of aerial mycelium was in the grey series and substrate mycelium was yellowish brown, no distinctive diffusible or melanoid pigments. Most of the tested carbon and nitrogen sources utilized has antifungal and slightly antibacterial activities. These properties of the isolate were nearest to the species *lydicus,* therefore the isolate N_{cu1} was suggested to belong to *Streptomyces* (Bergery's, 1984).

References

Abdel-Malek, Y. and Ishac, Y.Z. (1986) Evaluation of methods used in counting *Azotobacter. J. App Bacteriol.*, **31**, 267.

Benhamou, N. and Chet, I. (1993) Hyphal interaction between *Trichoderma harziomum* and *Rhizoctonia solani*. Ultrastructure and gold cytochemistry of the mycoparasitic process, *phytopathology*, **83** , 1062 .

Bergey's Manual of Determinative Bacteriology (1974) 8th ed. Buchanan. R.E. and Gibbons N.E. *Williams and Wilkins Company*, Baltimore, USA. 748, 752, 781, 794, 800-801, 303, 818-819.

Bergy's Manual of Systematic Bacteriology (1984) *Gram Negative Aerobic Rodsand Cocci* Vol. 1, Section 4, N.R. Krieg and J.G. Holt (Ed.), Williams & Wilkins, Baltimore, 220 – 229.

Burris, R.H., Okon, Y. and Albrecht, S.L. (1978) Properties and reaction of *Spirillum lipoferum*. Environmental role of nitrogen-fixing blue-green algae and asymbiotic bacteria. *Ecol., Stockholm*, **26**, 353.

Ceresini, P.C. and De-Souza, N.L. (1997) Association of binucleate *Rhizoctonia* spp. and *R. solani* kuhn AGuHGI and AG 2-2 III B to snap bean *(phaseolus vulgaris L.)* in Sao paullo state. *Summa-phytopthologica,* **23** (1), **14** .

Chamberlain, K. and Crawford, D.L. (1999) *In vitro* and *in vivo* antagonism of pathogenic turf grass fungi by *Streptomyces hygroscopicus* strains YCEDg and WYE53. *J. industrial. Microbiol. and biotechhnol.* **23** (1), 641.

Cho, J.Y. and Chung, S.J. (1998) Effect of rhizobacteria on the growth of cucumber and tomato Plug seedlings. *J. the korean society for horticultural-Science,* **39** (1), 18.

Cook, R.J. (1993) Making greater use of introduced microorganisms for biological control of plant pathogen. *Ann. Rev. phytopathol.* **31** , 53.

Dobereiner, J. (1978) Influence of envrionmental factors on the occurrence of *Spirillum lipoferum* in soil and roots. *Ecol. Bull. Stokholm,* **26,** 343.

El-Shanshoury, R.A. (1994) *Azotobacter chroococcum* and *Streptomyces atroolivaceus* as biocontrol agents of *xanthomonas compestris pr. malva cerum (cited from Rev. Plant patholo.,* 1995, **75** (4), 2295).

Gowily, Ahlam, E., Bouthina, F. Abdel-Ghany and Omar, S.A. (1993) Effect of biofertilizer application on controlling soybean root-rot disease. *Egypt. J. Appl. Soc.,* **(9),** 20 .

Gras, G. and Fernendez, F. (1993) Effect of application of filter cake and biofertilizers on vegetables cultivated on zeolite substrates Cultivos. *Tropicales,* **14** (2), 37.

Hasegawa, S., Kondo, N. and Kodaura, F. (1990) *Fusarium* wilt of adzukibean by rhizosphere microorganisms pest. *Alter.* 477.

Hassouna, M.G., El-Saedy, M.A.M. and Saleh, H.M.A. (1998) Biocontrol of Soil borne plant pathogens attacking cucumber (Cucumis) sativus by *Rhizobacteria* in a semiaried environment. *Aridsoil Research and Rehabilitation,* **12** (4), 345.

Jackson, M.L. (1958) *Soil Chemical Analysis.* Prentice-Hall, Inc., England, Cliffs, 219 – 221.

Kortemma, H.H. Rita, Haahtela and Smolander, A. (1994) Root-Colonization ability of antagonistic *Streptomyces grisiovirdis. Plant and soil.* **163** (1), 77.

Larson, R. (1987) Grading concerns rest control. How much is enough? *Market letter* 2. (Feb.) , 5.

Nemec, S., Dantoff, L.E. and Strandberg, J. (1996) Efficacy of biocontrol agents in planting mixes to colonize plant roots and control root diseases of vegetables and citrus. *Crop production,* **15** (8), 735.

Otten, W. and Gilligan, C.A. (1998) Effect of physical coniditions on the spatial and teusporal dynamics of the soil-borne fungal pathogen *Rhizoctonia solani. New phytologist,* **138** (4), 629.

Parameter, J.R.Jr. and Whitney, H.S. (1970) Taxonomy and nomenclature of the imperfect state. In : *Rhizoctonia solani. Biology and Pathology*, Parameter. J.R. (Ed) Univ. of California press USA ; 7 – 19.

Reddy, M.S., Campbell, S.E., Young, S.E. and Brown, G. (1991) Greenhouse evalution of rhizobacteria for the suppression of crown and root-rot of tomato caused by *Fusarium oxysporum* f. sp. radicis-lycopersici. *Bulletin SROP* **14** (8), 39.

Sanhita, G., Arora, D.K., Srivastava, A.K. and Gupta, S. (1995) Growth promotion of tomato plants by rihzobacteria and impsition of energy stress on *Rhizoctonia solani. Soil Biology and Biochem.* **27** (8), 1051.

Schloter, M. and Hartmann, A. (1998) Endophytic and surface colonization of wheat roots *(Triticum sativum)* by different *Azospirillium brasilense* strains studied with strain-specific monoclonal antibodies symbiosis-*Rehovot*, **25** (1 : 3), 159.

Schmiedekneckt, G. (1993) Biological control of *Rhizoctonia solani* kuhn on potatoes by microbial antagonists. *Archives of Phytopath. and plant protection*, **28**, (4) 331 .

Shende, S.T., Apte, R.G. and Singh, T. (1979) Influence of *Azotobacter* on germination of rice and cotton seeds. *Curr. Sci.* **46**, 675.

Shirling, E.B. and Gottlieb, D. (1966) Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacterio.* **16** (3) , 313.

Song, J.H., Yang, H.T., Gao, Q.A. and Wang, J.P. (1998) Preliminary studies on a strain of *Streptomyces* used in biocontrol. *Chiness J. biological control*, **14** (1), 18 .

Tahvonen, R. and Lahdenpeara, M.L. (1988) Biological control of *Botrytis cinerea* and *Rhizoctonia solani* in lettuce by *Streptomyces* sp. *Annales Agricul. fenniae*, **27** (2), 107.

Trego, E. and Ruben, S. (1997) Biological control and chemical studies on the antifungal compounds produced by *Streptomyces violaceusniger YCED.g PHD. Thesis*, Univ. of Idaho, U.S.A.

Waksman, S.A. (1959) Strain specificity and production of antibiotic substances X. characterization and classification of species within the *Streptomyces griseus* group. *Proc. Nat. J. Acad. Sci. U.S.A.* **45** , 1043 .

Waskman, S.A. and Lechevalier, H.A. (1962) *The Actinomycetes*. Vol. III. Antibiotics of Actinomycetes, Baltimore, the Williams and Wilkins Company, U.S.A. 430.

Weller, D.M. (1988) Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. phytopath.* **26**, 379 .

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دور بعض بكتريا وأكتينومايسيتات التربة فى مقاومة المسبب المرضى لمرض تعفن الجذور فى نبات الخيار

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تم اختيار ٢٥ عزلة من البكتيريا المثبتة للنتروجين، ١١ عزلة من *الأكتيومايسيتات* من عدد١١٧ عزلة تم عزلها من أراضى صحراوية وجذور نباتات مختلفة لدراسة تأثيرها على فطرة *الريزوكتونيا سولانى* المسببة لمرض تعفن الجذور.

وأوضحت النتائج أن عزلتى *الأزوتوباكتر* R$_{4}$ *والأزوسبريللم* Ke أفضل للعزلات تثبيتاً للنتروجين (٢١٠، ١٣٣ جزء من المليون) و زيادة لطول الجذر (٦١,٣%، ٦٥%) وكذلك تثبيطاً لنمو فطرة *الريزوكتونيا سولانى* (١٧,١٥ ملم) وقد صنفت العزلتين تصنيفاً كاملاً على أنهم *أزوتوباكتر كروكوكم وأزوسبيريللم ليبوفيرم*.

بالنسبة لعزلات *الأكتينومايسيتات*، كانت عزلة *الاستربتومايسس* N_{cu} أكفا العزلات قدرة على زيادة طول الجذر (٤١,٤%) وكذلك تثبيطا لنمو فطرة *الريزوكتونيا سولانى* (٢٢ملم). وقد صنفت العزلة تصنيفاً كاملاً على أنها *استربتومايسس لايديكس*.