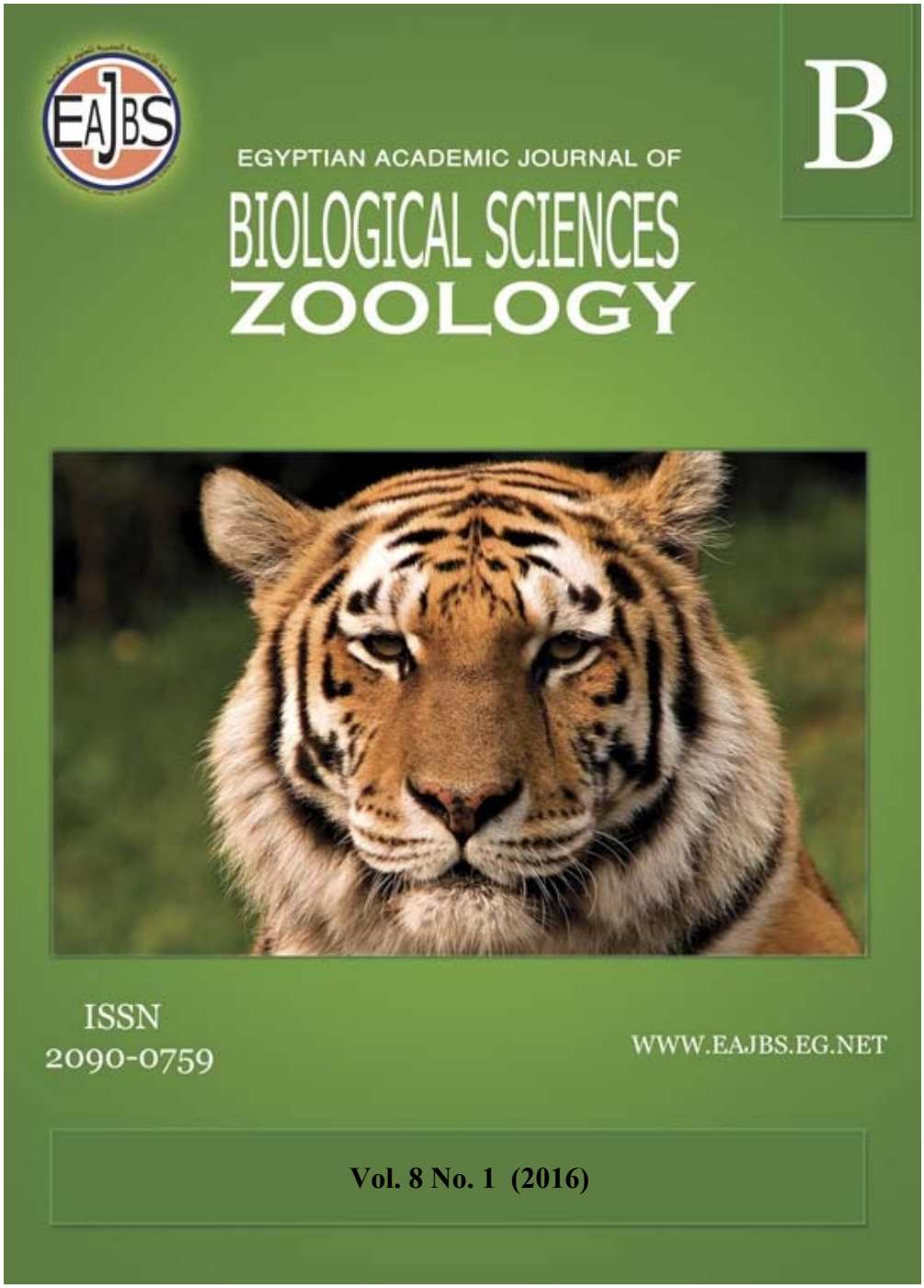

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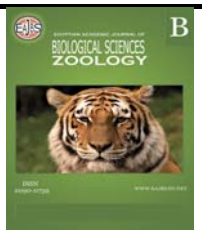

The Effect of *Serratia marcescens* and Genetically Improved *Pseudomonas fluorescens* on *Meloidogyne incognita*

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ABSTRACT

Pseudomonas fluorescens is an effective bacteria used in biocontrol agent for various soil borne plant diseases including plant parasitic nematodes. The aim of this study is enhancement the biocontrol effect of this bacterial strain by introducing chitinase gene(s) from *Serratia marcescens.* A DNA fragment containing the gene(s) were inserted into pGEM vector to construct a new plasmid, which introduced into *P. fluorescens.* Restriction enzyme digestion and chitin plate culture confirmed that the improved *P. fluorescens* is containing a functional chitinase gene(s). Bioassay comparative results among *P. fluorescens* wild type, *Serratia marcescens* and transformed *P. fluorescens* which conducted in screen house experiment indicated that transformed *P. fluorescens* had increased effect against plant parasitic nematodes (*Meloidogyne incognita).*

INTRODUCTION

Root-knot nematodes is one of the critical pests that causes fatal damage for wide range of important crops in tropical and subtropical regions (Zeinat *et al.* 2010). Endoparasites nematodes (*Meloidogyne* spp.) is the most common parasite nematodes in all over the world. Some of rhizosphere microorganisms are able to grow in root area and stimulate systemic defense against parasites including nematodes, this prevent pathogen to attach the plant and considering ideal tools for biocontrol agents. Both eukaryotic and prokaryotic microorganisms are widely present in the soil. Some of these have a potential for biocontrol of nematodes (Zaied *et al*., 2009, and Rehanadeh *et al*., 2013). The ability of *P. fluorescens* and *S. marcescens* to produce biological active ingredients have been reported in several studies (Shanthi, 1998; and Ramakrishnan, 1999) who find that, *Pseudomona fluorescens* as a biocontrol agent of *M. incognita*, and have a suppressing effect on nematodes multiplication and increase host plant growth. Microorganisms such as *Serratia marcescens* has been considered as a more natural and environmentally acceptable alternative to chemical control (Abd-Elgawad, 2006; and Zeinat *et al*. 2010).

Serratia sp. are short straight rods gram-negative bacteria, it found in soil, water and air. Their colonies are most often opaque. Somewhat iridescent and white, pink or red in color (Richared *et al.*, 1995). Chitenase has a greatest effect in biocontrol activity, (Gomaa, 2012). Chitenase could be suppressed egg hatching via deformed and destroyed the eggshell of *Meloidgyne* (Jung *et al*., 2002). In addition, more efficient bacteria that may able to control nematodes i.e have nematicidal activity, as well as maintaining the soil fertility via protoplast fusion between different species of *Peseudomones* and *Serratia.* Fusents were produced high mortality levels against nematodes (Zaied *et al*., 2009)

This study aim to 1- cloning of *S. marcescens* genes encoding the most abundant *S. marcescens* chitinase into both *E. coli* and *P. fluorescens*. 2-investigation of chitinase expression from various constructs of both *E. coli* and *P. fluorescens. 3-* improvement the transformed *P. fluorescens* as a biocontrol against plant parasitic nematodes, *M incognita*.

MATERIALS AND METHODS

The present study was carried out in plant protection department and Ain Shams Center for Genetic Engineering and Biotechnology (ACGEB), Faculty of Agriculture Ain Shams university.

A- **Materials**

1-Bacterial strains

The following bacterial strains were used:

Isolate numbe	Code	Identification	Source
1	ACGEB Sr.	*Serratia marcescens*	ACGEB
25	ACGEB Ps 11	*Pseudomonas fluorescence*	ACGEB
30	Top10	*Escherichia coli*	Qia gene

2-Host Plant Material

Screenhouse experiments were carried out using 21-day-old cucumber (*Cucumis sativus L.*) seedlings, (seeds of cucumber (Siena) were obtained from Kaha stations, Agriculture Research Center). They were transplanted singly in 20-cm-diam pots which filled with sterilized sandy clay soil.

3-Bacterial Media

1-Peptone Glycerol (PG): Peptone Glycerol (PG) 0.5% Bacto-peptone (Difco), 1.0% glycerol, 1.5% Bacto-agar (Difco), PH, 7.0. 2- King's (KB) was prepared according to King *et al*., (1954). 3- Luria broth (LB) was prepared according to King *et al*., (1954). 4- Chitin-amended Luria broth agar medium (LBCA) was prepared according to King *et al*., (1954) and used for culturing *Pseudomonas sp*.

4-Preparation of colloidal chitin

Colloidal chitin was prepared by partial hydrolysis of chitin (Sigma) with HCl 1M for 2 h at room temperature. The colloidal chitin was washed several times with large volumes of distilled water to eliminate all HCl and the pH was adjusted to 7.0.

5-Restriction enzyme reaction

Serratia DNA (10 µl), 10 X restriction enzyme buffer (2 µl), 10 U/ul EcoRI (2 µl), (4 µl) ddH_2O.

6-The ligation reaction

pGEM-T-Easy (0.05 ug/ul) (5 µl), Insert DNA (10 µl), 10X ligation buffer (2.2 µl), T4 DNA ligase (4 µl) and dd H_2O (0.8µl) and incubate overnight at 4°C.

7-Root-knot nematode culture:

Culture of *Meloidogyne incognita* (Mi) was maintained on tomato plants (*Lycopersicum esculentum* L.) growing in 20-cm pots in sterile sandy clay soil until needed, galled cucumber roots were washed with tap water, cutting into small pieces and placed in the mist chamber for egg hatching. The catch of the first day was excluded, then the following hatched IJs were collected daily and refrigerated at 5 ^{o}C for using.

B- Experimental methods:

1-Evaluation of bacterial activity on *Meloidogyne incognita* in greenhouse:

Pots filled with sterilized soil were divided into four groups (G1, G2, G3 and G4). The first group used as control without microbes (halve pots received 1000Mi as a positive control, the rest pots without Mi, were used as a negative control) All the other groups received microbes (one microbe/group) 10 ml/pot ($2.1x10^8$ cfu/ml). Half of the pots of each group was infected with Mi (1000 IJs/pot) and the other half was left Mi-free. All the tretments were replicated four times, each replicate was cultivated with three weeks seedling of cucumber (*Cucumis sativus L.*).

After 60 days screenhouse experiments were ended, the height of the plants was measured, fresh weight of roots and shoots was measured, and shoot/root ratio was calculated. IJs were extracted from roots after washing by mist chamber for 7 days, and from soil using modified Burmman funnel. All IJs which extracted were counted using light microscop. After that, roots were removed from the mist chamber and stained with acid fuchsin in cold lacto-phenol (McBeth *et al*., 1941). Galls were counted in the stained roots.

Survival bacteria in the soil was estimated after plant harvest by mixing 10g samples of soil from each pot of the bacterial treatment or control and estimating CFU per gram of soil by serial dilution plating on selective medium.

2-Chitinolytic activity; Agar medium screening test

Plates containing 0.5% acid swollen chitin was prepared. Two µl from each bacterial isolates was pipetted. After 2 days from incubation, the diameters in cm of the chitin hydrolysis zones were measured.

3-Chitinolytic activity

Chitinase activity was determined colorimetrically by detecting the amount of N-acetyl glucosamine (GlcNAc) released from a colloidal chitin substrate (Reissig *et al*. 1955).

4-Bacterial Transformation

Genomic library construction and screening

Chromosomal DNA was prepared from *S. marcescens* (ACGEB Ser1) according to the method described by Sambrook *et al*. (1989).

5-Preparation of Competent Bacterial Cells

Two hundred and fifty µl of 50 mM $CaCl_2$ was added into 2 tubes. The tubes was placed on ice, a sterile inoculating loop was used to aseptically transfer a single colony of bacteria from the starter plate to each tube. Ten µl of the ligation solution was added directly into the cell suspension in one of tubes. Ten µl (50 ng) of the control was added, linear, unligated pGEM T-Easy vector into the cell suspension of the second tube and returned to ice and incubated for 15 minutes.

6-Heat Shock of Bacterial Cells

Tubes was carried on ice to the water bath and transferred from ice and immediately immersed them into 42°C water bath for 90 seconds. It is critical that cells receive a sharp and distinct shock, immediately returned both tubes to ice and was leted them stand on ice for one additional minute and was placed in a test tube rack at room temperature. After that 250 µl of sterile liquid LB medium was added to

each tube. Gently the tubes was taped to mix to allow for the bacteria to recover from the heat shock at room temperature for about 30 minutes.

7-Plating the Transformation

Forty µl of 20-mg/ml X-Gal were spreaded onto each agar plate about 60 minutes before use, 100 µl of cells were pipetted onto each plate after that 4 µl of 1 M IPTG was added to the 100 µl of bacterial cells on the agar. Both together were spreaded onto the plate. A sterile cell spreader spread the bacteria was used over the surface of the agar in each plate. The spreader was moved back and forth on the agar while turning the plate. This will spread the bacteria evenly across the agar surface and placed the plates upside down in a 37°C incubator, and allowed them to grow for 24 to 48 hours. Alternatively, plates may be incubated at room temperature for 2 to 3 days, finally the number of blue colonies and white colonies on each plate was counted and recorded.

8-Plasmid Isolation

Single bacterial colony was transferred into 5 ml of LB according to Diab *et al*., (2002).

RESULTS

Chitinolytic activity; Agar medium screening test

The Gram-negative bacterium secretes a variety of extracellular enzymes including chitinases; *S. marcescens* is one of the most effective bacteria for degradation of chitin (Brurberg *et al.*, 2000). *Serratia* was subjected to test for their chitinase productivities as clear zone test for determination of the enzyme activity, Figure (1).

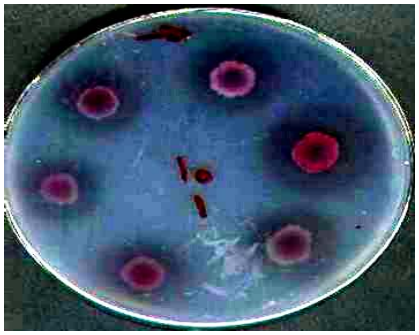

Fig. 1: Chitinase activity of *Serratia* strain as clear zone on chitin over lay plates.

Chitinolytic activity spectro-photometric assay

Chitinase activity was determined colorimetrically by detecting the amount of GlcNAc released from a colloidal chitin substrate (Reissig *et al*. 1955). *Serratia* strain gave the highest enzyme activity (1744.5 µg/m). Refaat *et al*, (1982) and Bechina *et al*, (1982) noted that, a correlation was not always observed between the largest zone with respect to activity on solid medium and the activity of the cultures on liquid medium (Table 1).

These data confirmed that, *E. coli* (pGEM 1) produces higher chitinase activity than the same strain containing pGEM2. Chitinase activities of the *P. fluorescens* ACGEB ps111 without transformed plasmid constructs were below the level of detection for this assay.

The genes encoding ChiB and CPB21 are linked, Gal *et al*., (1997) but the DNA sequence suggests that transcription of the two genes is not coupled. ChiB are found in the periplasm and/or culture medium of *S. marcescens* but this protein does not

contain typical N-terminal signal peptides. There are no indications of any proteolytic processing of ChiB Brurberg *et al.*, (1995).

Table 1: Concentrations of Chitinolytic activity of transformation measured by spectrophotometer

strains	concentration in micrograms per milliliter
S. marcescens ACGEBSer1	1728.0
*P. fluorescens*ACGEB ps111	Non detect
E.coli Top 10	Non detect
Transformed *E.coli* (PGEM 1)	960
Transformed *E.coli* (PGEM 2)	548
Transformed *E.coli* (PGEM 3)	456
Transformed *E.coli* (PGEM 4)	567
Transformed *P. fluorescens* ACGEB ps111 *(PGEM1)*	1067.9
Transformed *P. fluorescens* ACGEB ps111 *(PGEM2)*	753
Transformed *P. fluorescens* ACGEB ps111 *(PGEM3)*	Non detect
Transformed *P. fluorescens* ACGEB ps111 *(PGEM4)*	745

The chiB genes from *S. marcescens* have been transformed into other bacterial species like *P. fluorescens* and *E. coli* in an attempt to improve their ability to control fungal plant pathogens Sundheim *et al*, (1988) or to create new biocontrol agents.

Chitinolytic activity of transformed *E. coli* and *P. fluorescens*

The amount of N-acetyl glucosamine were determined colorimetrically by detecting released from a colloidal chitin substrate as described by Reissig *et al*. (1955). Results are shown in the previous table demonstrated that, the transformed *P. fluorescens* ACGEB ps111 *(PGEM1)* gave the highest enzyme activity (1067.9 µg/m) but *P. fluorescens* ACGEB ps111 *(pGEM3)* had no activity. The level of chitinase in Transformed *P. fluorescens* ACGEB ps111 *(pGEM1)* indicates that chitinase synthesis in these constructs is driven by *S. marcescenis* promoter that contained on the fragment insert, perhaps the chitinase promoter itself, and not from vector promoters. Clones producing significantly lower enzyme levels may therefore be undetectable and require a more sensitive screen. This conclusion is supported by Horwitz *et al*. (1984).

Cloning of a *S. marcescens* gene encoding chitinase

S. marcescens secretes chitinses of five distinct molecular masses of 58, 54, 52, 35, 22 kDa into the culture broth. *S. marces-cens* plasmid library was constructed using *E. coli* Top 10 by pGEM-T-Easy with *S. marcescens* ACGEB Ser1 chromosomal DNA. Overlay plates were used to identify chitinase producing clones. *S .marcescens* ACGEB Ser1 produced chitinolytic activity. Out of 320 clones screened, only four chitinase positive clones were identified by the production of clear zones, visible against the trans-lucent background of colloidal chitin.

A total of four independent clones (pGEM 1, 2, 3 and 4) express chitinase activity in *E. coli* Top10 on chitin overlay plates incubated at 30°C. *E. coli* Top 10 containing produces pGEM 1 (the largest zone of clearing), whereas the same strain containing pGEM 4 produces the smallest.

All four plasmid were mobilized into *P. fluorescens* ACGEB ps111, and the resultant isolate were analyzed for the rate of clearing on chitin overlay plates. In contrast to the results with *E. coli*, Out of 80 colonies screened on LB agar plates supplemented with 1% swollen chitin, three of them were found surround by chitin clear zone (Fig. 2).

The pGEM 1 construct produces the largest zone of clearing in *P. fluorescens* ACGEB Ps111 (Fig. 3), and the PGEM 3 construct produces the smallest However, *P. fluor-escens* ACGEB Ps111 (pGEM 2) required considerably longer time (>10 days) than *E. coli* (pGEM 1) 4 to 6 days to produce a detectable zone of clearing. This data

was conformed with Fuchs *et al.,* (1986). *In vitro* chitinase activities were determined for cultures of *E. coli* Top 10 and *P. fluorescens* ACGEB ps111 containing either no plasmid to quantitative the levels of chitinase produced.

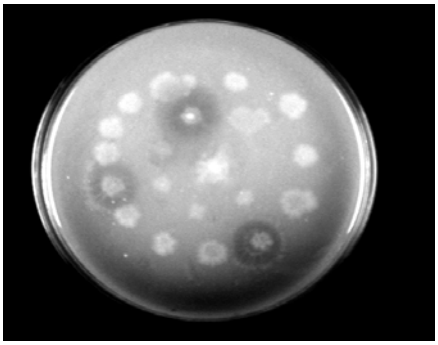

Fig. 2: Chitin clearance on chitin over lay plates by trans-formed *E. coli* (pGEM) after 4 days of incubation at 30°C.

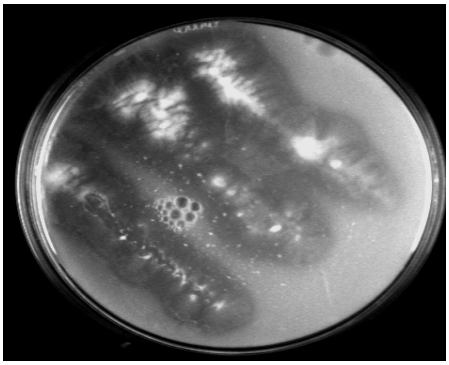

Fig. 3: Chitin clearance on chitin over lay plates by transformed *P. fluorescens* ACGEB ps111 *(pGEM)* after 4 days of incubation at 30°C.

Plasmid transfer to *E. coli* clone selection and expression

The vector containing the DNA fragment was transformed to *E. coli* top10, after transformation the bacteria was screened on LB plates containing Ampicillin, IPTG and XGAl and after 2 days only the white colonies were selected. Approximately 320 clonies were screened by transfer to LB agar plates supplemented with 1% swollen chitin. Out of 320 clonies screened, 4 clonies produced clear chitinolytic zones after 3 days of growth at 37^{O}C and were isolated. All clonies were chitinase positive, as judged by a clear zone around the colonies. The size of DNA insert was determined to be 6.5 kb.

Selection of transformed *P. fluorescens*

There is a narrow range of pH (about 12.0 - 12.5) which denatu-ration of linear DNA but not CCC-DNA occurs and that this property can be used for purifying CCC-DNA (Sharp *et al.,* 1972 and Currier and Nester 1976). The high concentration of sodium acetate causes precipitation of protein-SDS complexes (Kay *et al.,* 1952). Most of the three major contaminating macromolecules are co-precipitated and may be remo-ved by a single centrifugation in a bench top centrifuge. Plasmid DNA (and residual low molecular weight RNA) is recovered from the supernatant by ethanol precipita-tion. Plasmid DNA may be ana1yzed by gel electrophoresis either intact in the CCC form or after digestion with a restriction enzyme. After isolation of pGEM vector that contain ChiB insert, it retransformed to *P. fluorescens* ACGEB ps111.

The result of electrophoretic plasmid separation using 1.5% agarose gel electrophoresis is photographed (fig. 4). As shown from photo, (A) the transformed *E.*

coli pGEM contains the pGEM vector with Chi B fragment, (B) The Transformed *P. fluorescens* ACGEB ps111-PGEM with the same vector.

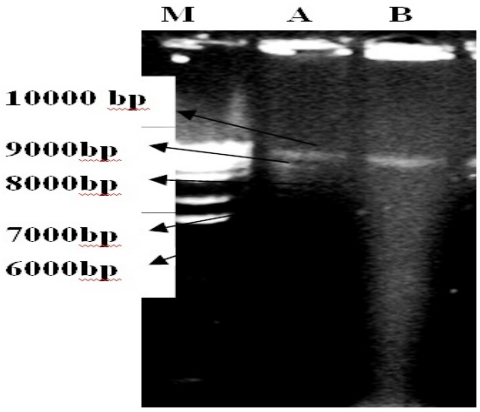

Fig. 4: Plasmid obtained from (A) Transformed *E.coli* pGEM and (B) *P. fluorescens* ACGEB ps111 pGEM.

Evaluation of *S. marcescenis*, *P. fluorescens* *and* transformed *P. fluorescens* activity on nematode infected soil.

Nematode parameters (number of galls, egg masses, and final population) were higher in unbacterized plants than on bacterized ones. Moreover, When the plants were inoculated with Mi only, significant suppression in the plant parameters (Shoot length, Shoot and Root Fresh weight, and shoot/root ratio) were recorded. Improving in these parameters was observed when adding different microbes (*Serratia marcescens, Pseudomonas fluorescens*, and transformed *P. fluorescens*) these data agreed with (Zaied *et al*., 2009).

Table (2) showed that: Maximum significant increasing in all the growth and yield parameters was found in treated plants by transformed *P. fluorescens* , *P. fluorescens and Serratia* as compared to untreated inoculated or other treatments.

Table 2: Effect of different bacterial strains (with and without Mi) on Cucumber growth parameters under greenhouse conditions.

treat	Plant hight	shoot	root	Shoot/root
Control (- Mi)	20.1^{e}	60.4^{e}	7.4^{ab}	8.2^{d}
	±1.877942	±3.603702	±0.648074	±1.238357
Control (+Mi)	16.2^{e}	52.4^{f}	4.5^{c}	11.6^{bc}
	±1.104536	±2.007209	±0.244949	±0.196964
Serratia marcescens	31.9^{cd}	82.8^{c}	6.3^{ab}	13.1^{ab}
	±1.790717	±1.959592	±0.374166	±0.496233
S. arcescens (+mi)	26.7^{d}	73.1^{d}	7.5^{a}	9.7^{cd}
	±1.306395	±1.551344	±0.163299	±0.41941
P. fluorescence	35.8^{c}	80.9^{c}	7.1^{ab}	11.3^{bc}
	±2.122891	±1.184155	±0.449691	±0.548839
P. fluorescence (+mi)	32.4^{cd}	76.9^{cd}	6.2^{b}	12.4^{b}
	±1.714643	±2.163844	±0.326599	±0.306457
T *P. fluorescence* (+mi)	56.9^{a}	104.2^{a}	6.9^{ab}	15.1^{a}
	±1.959592	±1.796292	±0.326599	±0.456254
Transform p+mi	47.6^{b}	90.5^{b}	7.2^{ab}	12.5^{b}
	±1.959592	±1.551344	±0.163299	±0.069679
f. value	118.08**	121.67**	14.34**	27.00**
l.s.d.	6.0941	7.2395	1.2773	1.9747

Numbers followed by the same letters did not differ significantly in their effects while, different letters had a statistically significant differences.

The obtained results indicated that, transformed *P.* is the most effective among all treatments in improving plant growth and reducing *Mi* population densities in soil.

In Table (3) results showed significant suppression of Mi., Maximum reduction in nematodes parameters was recorded with the transformed *P. fluorescens.*

Chitinases may have direct effect in nematode suppression (Elad *et al*., 1982). In the present study, the microbes which adding closed to the plant roots in large numbers in their media, that may contained toxins or by product of metabolites that may toxic to plant-parasitic nematodes and decrease other deleterious microorganisms (Khan and Saxena, 1997; Padgham and Sikora, 2007). Chitinase has a greatest effect in biocontrol activity (Gomaa, 2012), and it could be suppressed egg hatching via deformed and destroyed the eggshell of *Meloidgyne (*Duponnois, and Mateille, 1999; 4. Ali, 1996; and Mercer, *et al*., 1992). Moreover, it may be cause immature hatch of nematode eggs (Jung *et al*., 2002).

The use of *Serratia* may be able to control nematodes. This is in agreement with results reported by other researchers (Kurz *et al*., 2003, El-Nagdi and Youssef, 2004, Darby.2005, Zaied *et al*., 2009 and Zeinat *et al*., 2009).

Table 3: Effect of different bacterial strains on the root-knot nematodes *Meloidogyne incognita* infecting Cucumber under greenhouse conditions.

treat	pf	rr	golls	Egg masses
Control (+ mi)	4890.3[a]	4.89[a]	108.3[a]	87[a]
	±35.51838	±0.035518	±6.599663	±3.559026
S. arcescens (+mi)	760[b]	0.76[b]	43[b]	35.3[b]
	±21.22891	±0.021229	±3.265986	±2.054805
P. fluorescence (+mi)	640.3[c]	0.64[c]	32.3[b]	28[b]
	±12.65789	±0.012658	±2.054805	±2.160247
T *P. fluorescence* (+mi)	210[d]	0.21[d]	12[c]	9[c]
	±6.531973	±0.006532	±1.414214	±0.816497
f. value	20026.6**	20026.6**	229.55**	400.28**
l.s.d	70.07	0.0701	12.448	7.548

Numbers followed by the same letters did not differ significantly in their effects while, different letters had a statistically significant differences.

CONCLUSION

Each of the microbes: *S. marcescenis* , *P. fluorescens and* transformed *P. fluorescens Serattia* has a reducing effect on M. incognita but, the last one has the great effect in reduction followed by *P. fluorescens and S. marcescenis*.

All of these microbes recorded highly improving in plant growth.

So, we can use transformed *P.* as a bio control agent to control plant parasitic nematodes, and improving plant growth.

REFERENCES

AbAbd-Elgawad, M. M. M. and M.M. M. Mohamed (2006). Efficacy of selected bio-control agents on *Meloidogyne incognita* on eggplant. Nematologia mediterranea 34: 105-109.

AliA.H., (1996). Biocontrol of reniform and root- knot nematodes by new bacterial isolates. Bulletin of Faculty of Agriculture, University of Cairo, Egypt, 47(3): 487-497.

Bechina, E.M, L. G. Loginava, and M.V. Gernet. (1982). Selection of products of amylolytic enzymes among thermophilic Microorganisms. Prikl. Biokh-im. Microbial, 18: pp. 640-647.

Brurberg M. B., Bjørnar Synstad , Sonja Sletner Klemsdal , Daan M.F. van Aalten Leif Sundheim and Vincent G.H. Eijsink (2000). Chitinases from Serratia marcescens. Manuscript Recent Research Developments in Microbiology.

Brurberg, M.B., Eijsink, V.G.H. Haandrikman, A.J., Venema, G. and Nes, I.F. (1995). Chitinase B from Serratia marcescens BJL200 is exported to the periplasm without processing. Microbiology. 141:123-131.

Currier, T.C. and E.W. Nester (1976). Evidence for diverse types of large plasmids in tumor-inducing strains of Agro-bacterium. J. Bacteriol. 126 (1): 157-65.

Darby Creg . (2005) Interactions with microbial pathogens. Worm Book 6:1-15.

Diab, A.M; M.H. Abdel Aziz and S.A. Selim (2002). Plasmid encoded transferable antibiotic resistance in gram negative bacteria, Isolated from drinking Water in Ismailia City. Pakistan Journal of Biological Sciences, 5(7) pp. 774-779

Duponnois, R.BA.AM. And T. Mateille, (1999). Beneficial effect of Enterobacter cloacae and Pseudomonas mendocina for biological control of *Meloidogyne incognita* with the endospore-forming bacterium Pasteuria penetrans .Nematology, 1(1): 95-101

Elad, Y., Chet, I. and Henis, Y., ibid (1982). Degradation of plant pathogenic fungi by Trichoderma harzianum. Can. J . Microbial. 28:719-725.

El-Nagdi W. M. A. and Youssef M. M. A. (2004) Soaking faba bean seed in some bio-agents as prophylactic treatment for cont-rolling *Meloidogyne incognita* root-knot nematode infection. Pest science. 77: 2, 75-78.

Fuchs, R. L., Macphersosn, A . and Drahosd, J. (1986). Cloning of a S. marcescens gene encoding chitinase. Applied and Environ-mental Microbiology, 51:504-509.

Gal, S.W., Choi, J.Y., Kim, C.Y., Cheong, Y.H., Choi, Y.J., Bahk, J.D., Lee, S.Y. and Cho, M.J. (1997). Isolation and characteri-zation of the 54-kDa and 22-kDa chitinase genes of Serratia marcescens KCTC2172. FEMS Microbiol Lett.151: 197-204.

Gomaa E.Z. (2012). Chitinase production by B. thuringiensis and B. licheniformis: their potential in antifungal biocon-trol. J Microbiol; 50: 103−11.

Horwitz, M., J. Reid, and D. Ogaydziak (1984). Genetic improvement of chitinase pro-duction by S. mnarcescens. In J. P. Zikakis (ed.). Chitin. chitosan. and related enzymes. Academic Press Inc.. Orlando. Fla. pp.191-208.

Jung, Woo-Jin, Soon-Ju Jung, Kyu-Nam An, Yu-Lan Jin, Ro-Dong Park, Kil-Yong Kim, Bo-Kyoon Shon, And Tae-Hwan Kim (2002). Effect of Chitinase Producing Paenibacillus Illino-isensis KJA-424 on Egg Hat-ching of Root-Knot Nematode M. incognita. J. Microbiol. Biotechnol., 12(6), 865–871.

Kay, E.R.M.; N.S. Simmons and A.L. Dounce (1952). An Impro-ved Preparation of Sodium Desoxyribonucleate J. Am. Chem. Soc., 74: 1724-1726.

Khan, T. A. and S. K. Saxena. (1997). Effect of root-dip treatment with fungal filtrates on root penetration , development and reproduction of *Meloidogyne javanica* on tomato. International Journal of Nematology, 7: 85-88.

King, E.O., Ward, M.K. and D.E.Raney (1954). Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44 301-305.

Kurz, C.L., Chauvet, S., Andres, E., Aurouze, M., Vallet, I., Michel, G.P., Uh, M., Celli, J., Filloux, A. and De Bentzmann, S. (2003). Virulence factors of the

human opportunistic pathogen Serratia marcescens identified by in vivo screening. EMBO J. , 22: 1451–1460.

McBeth, C. W., A. L. Taylor and A. L. Smith (1941). Note on staining nematodes in root tissue. Proceedings of the Helminthological Society of Washington, 4: 18.

Mercer, C.F., D.R. Greenwood and J.L. Grant, (1992). Effect of plant and microbial chitinase on the eggs and juveniles of *Meloidogyne hapla* chitwood (nematode:Tylenchida). Nematologica, 38(2): 227-236.

Padgham, J. L. and R. A. Sikora (2007). Biological control potential and modes of action of Bacillus megaterium against *Meloidogyne graminicola* on rice. Crop Protection, 26: 971-977.

Ramakrishnan, S., C.V. Sivakumar, S.C. Dhawan and K.K. Kaushal, (1999). Biological control of rice nematode with Pseudomonas fluorescens proceeding of national symposium on national approaches in nematode management for sustainable agriculture, Anand, India, 23-25, November, 1998, 43-46.

Refaat, A. A., S.A. Ibrahim and M.A Borollosy (1982). Impro-vement of alphaamylase production by Aspergillus niger I. Mutagenic effect of N.T.G. Research Bulletin No. 1829, Ain Shams University.

Rehanandeh, G. Khodakaramin, N. Hassanzadeh, A. Seraji and S. M. Asghari (2013). Evaluation of antagonistic pseudomonas against root lesion nematode of tea. International J. of Biosciences, 3(3): p. 32-40.

Reissig JL, Strominger JL, Leloir LF (1955) A modification colo-rimetric method for the esti-mation of N-acetylamino sug-ars. J. Biol. Chem., 27:959–966

Richared, M.; Sambrook, B. and Frltsch, E. F. (1995). Direct transformation of s. marcsence. molecular bio technology., 4: 315-317

Sambrook, J., E.F. Fritsch and T. Maniatis (1989). Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Shanthi, A., S Raieswari, C.V. Sivakumar and U.K. Mehta, (1998). Soil application of Pseudomonas fluorescens for the control of root- knot nematode *Meloidogyne incognita* on grapevine (Vitis vinifera linn.) Nematology: Challenges and opportunities in 21 century. st Proceeding of the third international symposium of Afro-Asian Society of Nematologyists (TISAASN), Sugar-cane Breeding Institute (ICAR), Coimbatore, India, April 16-19: 203-206.

Sharp, P.A.; M.T. Hsu; E. Ohtsubo and N. Davidson (1972). Electron microscope hetero-duplex studies of sequence relations among plasmids of Escherichia coli. I. Structure of F-prime factors. J. Mol. Biol., 71(2):471-497.

Suarez, B., Rey, M., Castillo, P., Monte, E. and Llobell A. (2004). Isolation and characterization of PRA1, a trypsin-like protease from the biocontrol agent Trichoderma harzianum CECT 2413 displaying nematicidal activity. Applied Microbiological Biocontrol, 65:46-55.

Sundheim, L., Poplawski, A. R., and Ellingboe, A. H. (1988). Molecular cloning of two chitinase genes from Serratia marcescens and their expression in Pseudomonas species. Physiol. Mol. Plant Pathol., 33: 483–491.

Zaied, K.A.; Kawther, S. Kash; S.A. Ibrahim and T.M. Tawfik (2009). Improving Nematocidial Activity of Bacteria via Protoplast Fusion. Australian Journal of Basic and Applied Sciences, 3(2): 1412-1427.

Zeinat K, Mohammed SA, Radwan TEE, Hada GSAW. (2009). Potency Evaluation of Serratia marcescens and Pseudomonas fluorescensas biocontrol agents for root-knot nematodes in Egypt. Journal of Applied Sciences Research, 4(1): 93-102.

Zeinat K.M.; Nagwa, M. Atef; El-Sayed, S.A. and Abd El-Wahab G.S. (2010). Optimization of microbial biomass production as biocontrol agent against root knot nematode on faba plants. Journal of American Science, 6(6): 245-255.

ARABIC SUMMERY

تأثير بكتريا السيراتيا مارسيسينس و السيدوموناس فلوريسنس المحسنة وراثيا على نيماتودا تعقد الجذور

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يعتبر السيدوموناس فلوريسنس من البكتريا الفعالة فى المكافحة الحيوية لكائنات التربة الضارة بالنبات ومنها النيماتودا الممرضة للنبات.

والهدف من هذه الدراسة هو رفع كفاءة هذه البكتريا من خلال نقل الجين المسؤل عن افراز انزيم الكيتينيز من بكتريا السيراتيا مارسيسين الىها وذلك عن طريق:

نقل جزيئات ال DNA المحتوية على هذا الجين الى الناقل (PGEM) ثم ادخاله الى بكتريا ال السيدوموناس.

تم التأكد من نقل الانزيم وفاعليته عن طريق انزيمات القطع و اختبار بيئة صفائح الكيتين.

وللتأكد من كفاءة البكتريا المحسنة وراثيا بالمقارنة مع البكتريا الاصلية وبكتريا السيراتيا على نيماتودا تعقد الجذور على نباتات الخيار فى الصوبة.

كما أكدت نتائج الصوبة أن البكتريا المحسنة وراثيا كانت هى الاكثر كفاءة من الاصلية ومن السيراتيا.