Journal

MOLECULAR CHARACTERIZATION OF A "SUGAR BEET" *CUCUMBER MOSAIC CUCUMOVIRUS* ISOLATE AND ITS CONTROL *VIA* SOME PLANT GROWTH-PROMOTING RHIZOBACTERIA

Mona M. Oraby, A. M. El-Borollosy and M. H. Abdel-Ghaffar

J. Biol. Chem. Environ. Sci., 2008, Vol. 3(3): 147-167 www.acepsag.org

Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, P.O. Box 68, Hadayek Shubra 11241, Cairo, Egypt

ABSTRACT

During this investigation a *Cucumber mosaic cucumovirus* (CMV) was isolated from sugar beet (*Beta vulgaris* cv. Gazal) plants showing virus like symptoms, this was performed depending on specific polyclonal antibodies and *Chenopodium quinoa* as a local lesion host. Three of plant growth-promoting rhizobacteria were isolated from sugar beet plants rhizosphere, and identified morphologically and physiologically to be *Azotobacter chroococcum*, *Serratia marcescens* and *Pseudomonas fluorescens* species. Virus was purified from 200 gm of virus-infected *Nicotiana tabacum* cv. White Burley leaves giving $A_{260/280}$ ratio of 1.17 and a yield of 1.8 mg. Electron micrographs revealed spherical virus particles of about 30 nm in diameter. Virus coat protein (CP) molecular weight was estimated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE), giving 25 KDa band within resolving gel. Immunocapture-reverse transcriptase- polymerase chain reaction (IC-RT-PCR) was used for the amplification of CMV coat protein gene (*cp*), the appearance of 657 bp bands confirmed the expected size of such gene. Virus *cp* gene sequence and its alignment with the sequences of five overseas isolates confirmed that the isolate under study was related to the CMV serotype I. Sugar beet plants were treated by irrigation with bacterial crude cultures or their supernatant,

either separately or in the form of 1:1, 1:1:1 (v:v:v) mixtures. During treatment plants were inoculated with CMV, and studied for symptoms degree, virus presence, whole dry weights and root sugar contents two months post inoculation. Best results were obtained with 1:1:1 bacterial crude preparation as 10 plants out of 30 were found to be asymptomatic, and three of them confirmed to be virus-free using indirect enzyme-linked immunosorbant assay (I-ELISA) and IC-RT-PCR. Also these plants gave the highest growth measures of whole dry weight per plant (90.1 gm) and root sugar content (30 gm).

Keywords: *Beta vulgaris*, Plant growth-promoting rhizobacteria, *Azotobacter*, *Pseudomonas, Serratia*, *Cucumber mosaic cucumovirus*, *cp* gene, Sequencing, I-ELISA, IC-RT-PCR, Sugar content.

INTRODUCTION

Egypt's sugar industry is turning to sugar beet (*Beta vulgaris*) to achieve the long-term goal of self-sufficiency, while trying to improve the efficiency of its sugar cane crop which suffers from many problems. The cultivated area from sugar beet in Egypt is 170000 feddans in 2006 with average yield of 22 tons per feddan (FAO, 2006).

The major viruses that infect sugar beet worldwide and affect its production are *Cucumber mosaic virus* (CMV) (El-Kady *et al*, 1985; Abdel-Salam *et al*, 1997; Xi *et al*, 2006) and *Beet mosaic potyvirus* (BtMV) (Abdel-Ghaffar *et al*, 2003; Stevens, 2007).

CMV, genus: *Cucumovirus*, family: Bromoviridae, is one of the most widespread plant viruses with extensive host range infecting about 1000 species including cereals, fruits, vegetables and ornamentals (Roossinck, 1999). The virus is readily transmitted in a non-persistent manner by more than 75 species of aphids (Palukaitis *et al*, 1992). CMV is a multicomponent virus with a single stranded positive sense RNA. RNAs 1 and 2 are associated with viral genome replication while RNA 3 encodes for movement protein and coat protein. Numerous strains of CMV have been classified into two major subgroups (subgroups I and II) on the basis of serological properties and nucleotide sequence homology (Palukaitis *et al*, 1992; Madhubala *et al*, 2005). The subgroup I has been further divided into

two groups (IA and IB) by phylogenetic analysis (Roossinck *et al*, 1999).

Pathogenic microorganisms affecting plant health are a major and chronic threat to food production and ecosystem stability worldwide. However, increasing use of chemical inputs causes several negative effects (Gerhardson, 2002). Furthermore, the growing cost of pesticides and consumer demand for pesticide-free food has led to a search for substitutes for these products. There are also a number of fastidious diseases, mainly viral and viroid diseases, for which chemical solutions are ineffective (Shehata and El-Borollosy, 2008). Plant growth-promoting bacteria (PGPB) are associated with many, if not all, plant species and are commonly present in many environments. The most widely studied group of PGPB are plant growth-promoting rhizobacteria (PGPR) colonizing the root surfaces (Kloepper *et al*, 1999), i.e., *Azotobacter*, *Azospirillum*, *Rhizobium*, *Bacillus*, *Pseudomonas* and *Serratia* (Compant *et al*, 2005).

Many investigators studied the effect of PGPR on controlling plant virus's infection. Bergstrom *et al* (1982) showed that resistance in cucumber against CMV could be induced by previous treating of plants with *Colletotrichum orbiculare* or *Pseudomonas syringae*. Maurhofer *et al* (1994) studied successfully the effect of *P. fluorescens* on the resistance of tobacco against *Tobacco necrosis virus* (TNV). De Meyer *et al* (1999) enhanced the resistance of tobacco plants against *Tobacco mosaic virus* (TMV) using *P. aeruginosa* and Ryu *et al* (2004) protected *Arabidopsis thaliana* plants against CMV infection using *S. marcescens*. Shehata and El-Borollosy (2008) studied the effect of treating squash plants with some isolates of PGPR (*B. subtilis, P. fluorescens and S. marcescens)*, on controlling *Zucchini yellow mosaic potyvirus* (ZYMV) infection and enhancement of plants growth.

The present investigation aimed to isolate CMV from sugar beet plants, and identifying it depending on some molecular characteristics. Isolation and identification of some PGPR and their application for controlling CMV infection within sugar beet plants were also studied.

MATERIALS AND METHODS

Virus and bacteria isolation

Cucumber mosaic cucumovirus was isolated from sugar beet plants (*B. vulgaris* cv. Gazal) showing virus-like symptoms (collected from the open fields of Kafer El-Sheikh Governorate, Egypt). Isolation was performed depending on I-ELISA according to Koenig (1981) using specific polyclonal antibodies (Agdia Inc., USA), and *Chenopodium quinoa* as local lesion hosts. Virus was maintained on *Nicotiana tabacum* cv. White Burley under greenhouse conditions (28ºC ±2).

On the other hand, three of sugar beet rhizosphere bacteria were isolated depending on the agar plate dilution method according to Black (1996). Media used for isolation were nutrient agar, King's medium B (KMB) and nitrogen–free Jensen's medium (Sigma-Aldrich Inc., USA).

Bacterial cultures

Three isolates of sugar beet rhizosphere bacteria were isolated as pure cultures. Bacterial colonies properties were determined, and cells were observed under microscope after Gram and spore staining. Essential biochemical tests were carried out according to Collins and Lyne (1984). Moreover, bacteria were identified according to Farmer (1984), Holt *et al* (1994) and Ahmad *et al* (2005).

Bacteria were grown separately in nutrient broth or Ashby's mannitol media for 72 h/30ºC on a shaker (200 rpm). Crude cultures or their supernatant (after centrifugation at 5000 rpm/ 30 min) were used directly for plants treatment.

Virus purification

Leaves of *N. tabacum* cv. White Burley (200 gm) showing severe symptoms were harvested two weeks post inoculation, kept at -20ºC overnight and used for virus purification. Virus particles were purified using a modified protocol of Lot *et al* (1972) as described by El-Afifi *et al* (2007). The virus yield was determined by considering A_{260} 5.0 = 1 mg/ml (Sarma *et al*, 2001). Preparations were negatively stained with 2% phosphotungastic acid (PTA) according to Griffin (1990) and examined with a Philips EM400T transmission electron microscope, Specialized Hospital, Ain Shams University, Cairo, Egypt.

SDS-PAGE of CMV coat protein

The molecular weight of CP subunits of CMV was determined by SDS-PAGE using 4% stacking gel on a 12% resolving gel and the buffer system as described by Laemmli (1970) and Shukla and Ward (1988).

IC-RT-PCR for CMV *cp* gene amplification

The IC-RT-PCR was performed using infected tobacco leaf collected 15 days post CMV inoculation. Immunocapturing and cDNA synthesis was carried out as described by Minafera and Hadidi (1994). The following primers (Invitrogen Corp., USA) were used for the amplification of CMV *cp* gene were designed depending on CMV *cp* gene sequences collected from PubMed (GeneBank) web site (http://www.ncbi.nlm.nih.gov): 5'ATGGACAAATCTGAATCAAC3' (Sense) and 5'TCAAACTGGGAGCACCCCAG3' (Antisense).

PCR was performed as described by Ghosh *et al* (2002) using PerkinElmer Cetus Thermal Cycler PE 9700 (PerkinElmer Inc., USA). Five µl from resulting cDNA were transferred to tube containing 45 µl PCR reaction mixture. PCR program was 94°C initial melting for 3 min followed by 35 cycles of 94°C/1 min, 55°C/1 min, 72°C/2 min and 72°C/10 min final extension.

For PCR product analysis, 1.5% agarose gel was used and electrophoresis was carried out in Sub-Cell DNA apparatus (Bio-Rad® Lab., USA) at 80 V. The amplified gene band was visualized on an UV Transilluminator and photographed by Gel Documentation System (AlphaImager® TM1220, Documentation and Analysis system, Canada).

Purification of PCR product

DNA fragments representing viral *cp* gene were purified from agarose gel using the gel slicing and melting method described by Wieslander (1979). The final pellets were washed with 1 ml of 70% ethanol, then left to dry in air and resuspended in 30 µl TE buffer and stored at -20°C.

CMV *cp* gene cloning, sequencing and sequence analyses

The *cp* genes were ligated into pGEM®-T vector plasmids, which were used for *Escherichia coli* JM109 competent cells transformation according to Promega's pGEM®-T Easy Cloning kit manual's instructions. Sequencing was carried out at Gene Analysis

Unit (VACSERA, Agouza, Cairo, Egypt) using ABI Prism® BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA). Sequencing products were resolved in an automated sequencer model 310 (Applied Biosystems, USA).

DNA sequences of CMV *cp* genes from different worldwide isolates were collected from GeneBank (http://www.ncbi.nlm.nih.gov). Strains were from countries and with accession numbers as follows: Chinese, AJ006990; Korean, AB109908; Japanese, AB070622; Indian, EU573928 & USA, U31220. These sequences were aligned with the nucleotide sequence of the under study CMV isolate using the DNAsis Max Software (Helixx Technologies Inc., Canada). Phylogenic tree and similarity were performed using MEGA 4 software (http://www.megasoftware.net).

Plants treatment, viral infection and growth evaluation

Treatment of sugar beet plants with either crude bacterial cultures (72 h age) or their supernatant was carried out as follows:

Healthy seedlings (each carrying 4 leaves and planted in 30 cm diameter pot containing sterilized planting mixture of 1:1:1 clay: sand: peat moss, w:w:w) were used. Plants were irrigated with 200 ml from each of the three crude bacterial cultures or their supernatant, either separately or as 1:1 or 1:1:1(v:v:v) mixture (30 plants were used for each treatment). The irrigation was done with preparations every week for one month, and if soils were dried out it was performed using distilled water. After that period from bacterial treatment plants were mechanically inoculated with CMV, and tested for symptoms degree, virus presence two months later (without stopping treatments) using I-ELISA and IC-RT-PCR. Also dry weights, root sugar contents (sugar was determined depending on the method of Le-Docte (1927) using an automatic saccharimeter) were determined to evaluate the effect of bacterial treatments on plants growth and virus infection controlling. Note that control healthy plants were always irrigated with water, while infected controls were inoculated with virus and left without any treatment.

RESULTS AND DISCUSSION

Results:

Isolation of virus and bacteria

CMV was isolated from naturally infected sugar beet plants (showing mosaic and malformation) (Figure 1). Samples which gave positive I-ELISA results with CMV specific antiserum produced chlorotic local lesions on *C. quinoa*. Three cycles of local lesions isolation were performed and the last produced lesions were inoculated on *N. tabacum* cv. White Burley plants.

Figure 1. Sugar beet plant used for CMV isolation showing mosaic, mottling and malformation.

On the other hand, three bacterial cultures were isolated from sugar beet rhizosphere and identified to the degree of genus depending on colonies, morphological and physiological characteristics (Table 1). Results obviously showed that isolate no. 1 tends to be similar in characters to genus *Azotobacter* and towards *A. chroococcum*. While isolate no. 2 considered as *Pseudomonas fluorescens* and isolate no. 3 was confirmed to be *Serratia marcescens*.

Table 1. Differential characteristics of the isolated rhizobacteria

Characteristics & Biochemical tests		Isolate number		
		Azotobacter	*Pseudomonas*	*Serratia*
Morphology		Cocci in pairs	Short rods	Short rods
Spores		+	-	-
Gram staining		-	-	-
Capsule		+	-	-
Motility		Motile	Motile by polar flagellum	Motile
Pigments		Brownish undifusible	Fluorescent bluish diffusible	Red undifusible
Oxygen requirements		Aerobic	Aerobic	Aerobic & facultatively anaerobic
Growth temp. (ºC):	4	-	+	-
	30	+	+	+
	37	-	+	+
	41	-	+	-
	50	-	ND	-
Growth pH:	4	-	-	±
	5.7	+	+	-
	6.8	+	+	±
	9.0	+	-	-
Growth in NaCl (%):	5	±	+	-
	7	-	-	-
	10	-	-	-
Gelatin hydrolysis		+	-	+
Starch hydrolysis		+	-	-
Glucose		+	+	+
Maltose		+	-	+
Manitol		+	-	+
Sucrose		+	±	+
Citrate		+	+	+
Voges Proskauers (VP)		+	ND	+
Indol		+	-	-
Nitrate reduction		+	+	-

+: Positive reaction or Good growth, ±: Moderate growth, -: No growth and ND: Not detected.

Virus purification

UV absorption data show that $A_{260/280}$ ratio for purified virus preparation was 1.17, while yield was 1.8 mg virus/200 g infected leaves. Electron micrographs show spherical virus particles of about 30 nm in diameter (Figure 2).

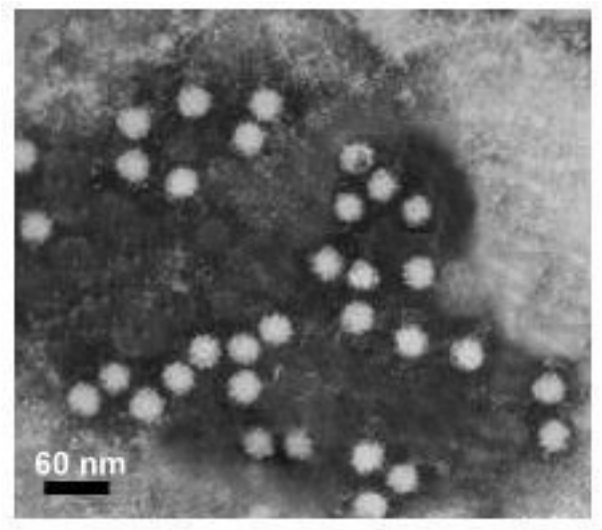

Figure 2. Electron micrograph of negatively stained purified CMV preparation.

Molecular weight of CMV CP

Results in Figure (3) showed that the SDS-PAGE analysis of the purified CMV preparation indicating the CMV CP. The protein appeared as one band with a molecular weight of about 25 KDa .

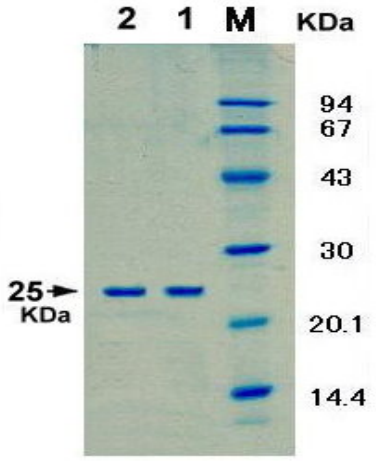

Figure 3. SDS-PAGE of purified CMV preparation (Lanes 1 and 2). M: Protein marker (Promega, USA).

IC-RT-PCR for CMV *cp* gene amplification

The agarose gel analysis of the PCR product indicated a single band with size length of 657 bp which is the expected size for the CMV *cp* gene Figure (4).

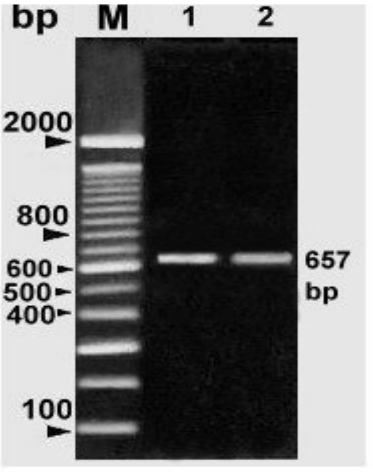

Figure 4. IC-RT-PCR for the amplification of CMV *cp* gene, lane 1 & 2. Lane M: DNA Ladder (Promega , USA).

CMV *cp* gene sequencing and sequence analyses

The nucleotide sequence of CMV *cp* proved that it is consists of 657 base, started with ATG and ended with TTG, as start and stop codons, respectively. Sequence was aligned with five overseas isolates (Figure 5), the alignment was converted to phylogenetic tree (Figure 6). The Egyptian CMV isolate of this study proved 94 % similarity with the Chinese and Indian isolates (which are belonging to serotype or subgroup I), 93 % & 91 % was found with the Japanese and USA isolates (subgroup 1B), respectively. On the other hand, 71 % similarity was obtained with the Korean isolate (serotype II).

Evaluation of plants induced resistance and growth

Biologically: Sugar beet infected and control plants were studied for symptoms development and degrees. Results are demonstrated in Table (2) proving that *Azotobacter:Serratia:Pseudomonas* crude cultures 1:1:1 (v:v:v) mixture treatment was the best as 10 plants out of 30 were asymptomatic, followed by *Azotobacter:Serratia* 1:1 crude cultures mixture which gave 7 asymptomatic plants. All of the 30 untreated control plants gave viral severe symptoms.

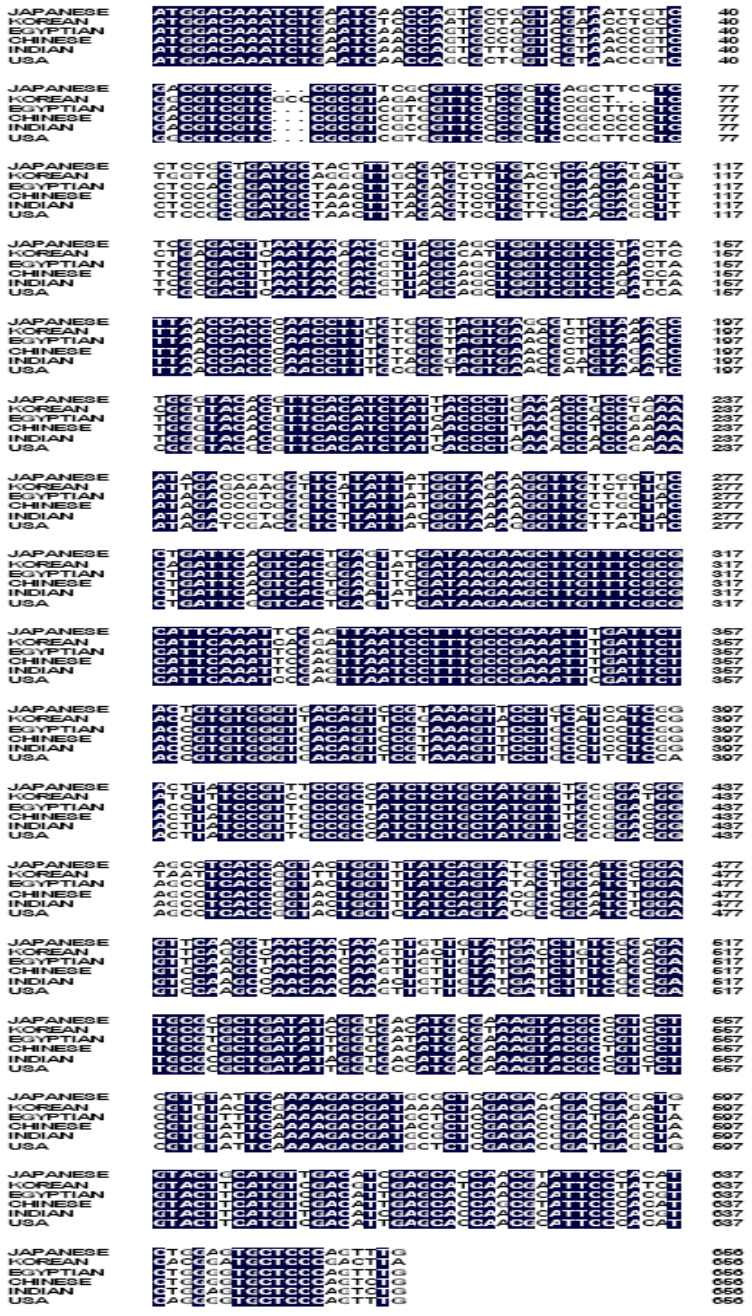

Figure 5. Multiple sequences alignment for CMV Egyptian isolate *cp* gene with other CMV isolates representing different subgroups.

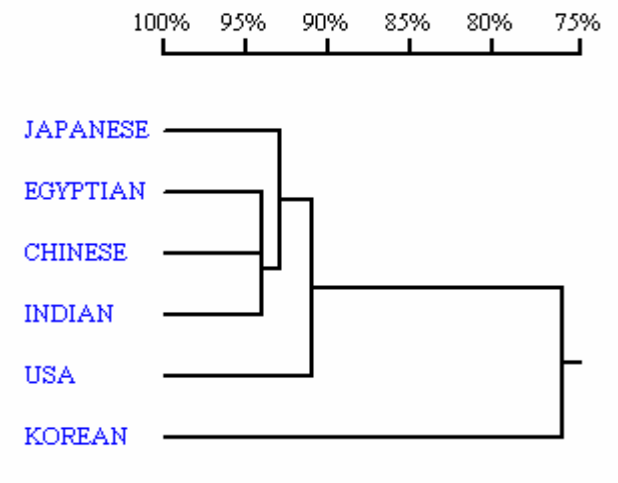

Figure 6. Phylogenetic tree between the Egyptian CMV of this study and five overseas isolates.

Table 2. Effect of rhizobacteria treatments on sugar beet CMV symptoms.

Bacterial treatments*	Crude			Supernatant		
	Severe	Mild	No	Severe	Mild	No
1	10	17	3	13	17	0
2	14	14	2	18	12	0
3	10	16	4	14	16	0
4	9	16	5	10	17	3
5	8	15	7	10	15	5
6	11	14	5	14	16	0
7	5	15	10	8	16	6

* Thirty plants were treated with either crude bacterial cultures or their supernatant for each treatment and tested for viral symptoms two months post inoculation.
1= *Azotobacter*, 2= *Pseudomonas*, 3= *Serratia*, 4= 1+2 (1:1), 5= 1+3 (1:1), 6= 2+3 (1:1) & 7= 1+2+3 (1:1:1).
Note: All of the thirty control plants produced severe symptoms.

I-ELISA and PCR: As best results were obtained from plants treated with bacterial crude cultures, plants giving no symptoms from these treatments were selected for I-ELISA test. For confirmation plants which gave negative I-ELISA results were further tested with PCR.

Data in Table (3) revealed that 4 out of 10 asymptomatic plants, treated with 1:1:1 bacterial crude cultures mixture, gave negative I-ELISA values, 3 of them proved to be virus-free by PCR.

Table 3. I-ELISA values and PCR results for symptomless sugar beet plants treated by bacterial crude cultures.

Treatments		ELISA values and PCR results									
		1	2	3	4	5	6	7	8	9	10
1	E	0.311 –	0.932 +	0.841 +							
	P	+									
2	E	1.051 +	0.890 +								
	P										
3	E	0.251 –	0.741 +	0.666 +	0.911 +						
	P	+									
4	E	0.149 –	0.300 –	0.844 +	0.891 +	0.922 +					
	P	–	+								
5	E	0.222 –	0.314 –	0.210 –	0.561 +	0.711 +	0.841 +	0.688 +			
	P	–	+	–							
6	E	0.299 –	0.901 +	0.888 +	0.946 +	0.998 +					
	P	+									
7	E	0.241 –	0.301 –	0.211 –	0.316 –	0.755 +	0.718 +	0.900 +	0.615 +	0.700 +	0.518 +
	P	–	+	–	–						
Controls		Healthy	Infected								
	E	0.129 –	1.062 +								
	P	–	+								

E: ELISA value, P: PCR result, +: positive & –: negative.
Each ELISA value was the average of three numbers.
1= *Azotobacter*, 2= *Pseudomonas*, 3= *Serratia*, 4= 1+2 (1:1), 5= 1+3 (1:1), 6= 2+3 (1:1) & 7= 1+2+3 (1:1:1).
Note: Plants giving positive ELISA results were not tested with PCR.

Plant growth: Three plants giving different symptoms degrees from different bacterial crude cultures treatments were tested for dry weights and root sugar contents compared with infected and healthy controls. Data in **Table (4)** revealed that 1:1:1 mixture treatment gave the highest growth measures and sugar contents values for the asymptomatic plants, reaching 90.1 & 30 gm for dry weight per plant and root sugar, respectively.

Table 4. Effect of bacterial treatments on sugar beet CMV infection resistance as described by dry weight and root sugar content

Bacterial treatments		Plants* from crude bacterial culture treatments showing different symptoms								
		Severe			Mild			No		
		1	2	3	1	2	3	1	2	3
1	DW	50.9	52.8	60.1	57.1	55.1	61.5	60.1	59.5	65.1
	SC	9.5	10.1	12.4	10.8	10.0	9.0	17.5	15.2	18.5
2	DW	40.9	45.1	50.1	50.2	52.1	49.1	50.5	66.7	-
	SC	7.5	8.9	10.9	8.3	9.4	7.9	12.3	17.9	-
3	DW	50.4	56.1	49.9	60.2	64.3	63.2	70.1	66.4	69.5
	SC	9.1	10.9	8.7	16.5	11.9	10.5	19.4	16.9	19.5
4	DW	48.1	55.7	58.5	55.9	60.1	58.9	72.3	80.2	85.2
	SC	8.3	9.9	11.5	10.9	13.4	11.5	21.4	23.1	22.4
5	DW	61.4	64.2	59.1	70.3	69.5	70.9	75.4	80.5	79.5
	SC	15.2	15.9	12.3	18.9	17.2	20.5	24.2	27.8	26.7
6	DW	55.4	57.2	60.1	69.8	66.9	70.1	70.7	69.1	72.5
	SC	9.8	10.5	12.0	15.8	15.0	19.2	23.5	20.5	21.3
7	DW	63.3	67.6	55.4	71.0	75.2	71.6	81.5	89.6	90.1
	SC	16.0	16.9	11.5	20.9	22.3	19.5	28.2	29.0	30.0
Controls		Healthy			Infected					
	DW	90.0	79.3	92.1	50.4	45.0	60.2			
	SC	30.5	27.1	33.0	15.2	12.7	14.0			

* Three replicates were selected from each symptoms degree.
1= *Azotobacter*, 2= *Pseudomonas*, 3= *Serratia*, 4= 1+2 (1:1), 5= 1+3 (1:1), 6= 2+3 (1:1) & 7= 1+2+3 (1:1:1).
DW= Whole plant dry weight & SC= Root sugar contents (in gm).

Discussion

Sugar beet ranks the second important sugar crop after sugarcane, producing annually about 40 % of sugar production all over the world (FAO, 2006). Despite the newness of sugar beet in Egypt, it has a large importance where there are wide newly reclaimed sandy soils at the northern parts of Egypt, which could be cultivated with sugar beet in addition to its ability to produce high yields of sugar under saline soil conditions. The Egyptian Government is also interested in using such sugar source instead of sugarcane due to its high water consumption (Leilah *et al*, 2005).

During the present investigation CMV was isolated from sugar beet plants showing mosaic, mottling and malformation. The virus was confirmed by specific antiserum and ELISA, also, electron micrographs of the purified preparation revealed spherical particles

with 30 nm in diameter. Results were in harmony with Madhubala *et al* (2005) and El-Afifi *et al* (2007).

Three of PGPR were isolated from sugar beet rhizosphere and confirmed to be *A. chroococcum*, *S. marcescens* and *P. fluorescens* depending on some physiological tests, their colonies and morphological characteristics. The obtained results were compared with what found by Farmer (1984), Collins and Lyne (1984), Holt *et al* (1994), Ahmad *et al* (2005) and found to be in agreement.

The virus was molecularly characterized, and with the aid of SDS-PAGE found to has a CP with molecular weight of 25 KDa. Also, IC-RT-PCR was used for virus *cp* gene amplification, which produced band with a size of about 657 bp, results which were similar to those obtained by Madhubala *et al* (2005) who worked on the characterization of CMV Vanilla (*Vanilla planifolia* Andrews) isolate from India.

The present isolate gave 94 % similarity with the Chinese (AJ006990) and Indian (EU573928) isolates (belonging to serotype I), 93 % & 91 % with the Japanese (AB070622) and USA (U31220) isolates (subgroup 1B), respectively. On the other hand, 71 % of similarity was obtained with the Korean (AB109908) isolate (serotype II). Madhubala *et al* (2005) and Verma *et al* (2006) characterized CMV from Vanilla and Geraniums (*Pelargonium* spp.) plants, respectively, depending on molecular characteristics. They isolated, cloned and sequenced *cp* gene, and aligned the nucleotide and amino acid sequences with other worldwide CMV isolates belonging to different subgroups.

Sugar beet plants treated by irrigation with bacterial crude cultures or their supernatant were subjected to CMV infection. The best results were obtained with plants treated with 1:1:1 crude bacterial cultures mixture, as 10 out of 30 were asymptomatic, 4 & 3 of them gave negative results by ELISA and IC-RT-PCR, respectively. Those plants had the higher dry weights and sugar contents, i.e., 90.1 per plant & 30 gm per root, respectively.

PGPR-mediated induced resistance was reported against TNV (Maurhofer *et al*, 1994) and TMV (De Meyer *et al*, 1999) in tobacco. In each case, a single PGPR strain of *P. fluorescens* was used as the inducing agent, and the virus infection resulted in a local lesion response so resistance was evaluated as a reduction in lesions number and size.

Murphy *et al* (2003) evaluated the combinations of two strains of PGPR (*Bacillus* sp.) formulated with the carrier chitosan for the ability to induce growth promotion of tomato plants and resistance to infection by CMV. When plants were challenged with CMV, all plants in the biopreparation treatments and the older control treatment had significantly greater height, fresh weight, and flower and fruit numbers than that of plants in the CMV-inoculated same age control treatment. Shehata and El-Borollosy (2008) tested *B. subtilis*, *S. marcescens* and *P. fluorescens* liquid crude cultures and their Millipore® filtrates for their ability to induce systemic resistance within squash plants (*Cucurbita pepo* var. Eskandarany) against ZYMV infection. Data proved that best results were obtained by treatment of seeds germination with the *Serratia* crude culture for 72 h, as number of symptomless plants were 9 out of 30 plants inoculated, followed by *Pseudomonas* treated plants which gave 7 asymptomatic plants. The induced resistance was tested using I-ELISA and IC-RT-PCR for the detection of ZYMV helper component proteinase gene (*Hc-pro*), which proved that the mentioned symptomless plant were virus-free or with a low concentration of virus infection.

REFERENCES

Abdel-Ghaffar, M.H.; M.I. Salama and S.Y.M. Mahmoud (2003). Electron microscopy, serological and molecular studies on an Egyptian isolate of *Beet mosaic potyvirus*. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo. 11: 469-484.

Abdel-Salam, A.M.; A.A. Hassan; M.M. Merghany; K.M. Abdel Ati and Y.A. Ahmed (1997). The involvement of a geminivirus, a closterovirus, and a spherical virus in the interveinal mottling and yellows diseases of cucurbits in Egypt. Bull. Fac. Agric. Univ. of Cairo 48: 707-722.

Ahmad, F; I. Ahmad and M.S. Khan (2005). Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent *Pseudomonas* in the presence and absence of tryptophan. Turk. J. Biol. 29: 29-34.

Bergstorm, G.C.; M.C. Johnson and J. Kuc (1982). Effect of local infection of cucumber by *Colletotrichum lagenarium*, *Pseudomonas lachrymans*, or *Tobacco necrosis virus* on systemic resistance to *Cucumber mosaic virus*. Phytopathology 72: 922-926.

Black, J.G. (1996). Microbiology: Principles and Applications, 3rd Edition, pp. 140-144. Prentice Hall, Upper Saddle River, New Jersey, USA.

Collins, C.H. and P.M. Lyne (1984). Microbiological Methods, (5th Ed.), pp. 56-113. Butterworths Co. Ltd., London.

Compant, S.; B. Duffy; J. Nowak; C. Clement and E. Ait Barka (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Applied Envirol. Microbiol. 71: 4951-4959.

De Meyer, G.; K. Audenaert and M. Hofte (1999). *Pseudomonas aeruginosa* 7NSK2-induced systemic resistance in tobacco depends on in planta salicylic acid but is not associated with PR1a expression. Eur. J. Plant Pathol. 105: 513-517.

El-Afifi, Sohair I.; A.M. El-Borollosy and S.Y.M. Mahmoud (2007). Tobacco callus culture as a propagating medium for *Cucumber mosaic cucumovirus*. Inter. J. Virology. 3: 73-79.

El-Kady, M.S.; A.S. Gamal El-Din; M.K. Nakhla and A.M. Abdel-Salam (1985). Strain of CMV isolated from sugar beet in Egypt. The 1st Nat. Conf. of Pests and Dis. of Veg. and Fruits in Egypt, Suez Canal Univ., Ismailia, Egypt, pp. 617-626.

FAO, Food and Agriculture Organization of the United Nations, Rome, 2006.

Farmer, J.J., (1984). Other genera of the family Enterobacteriaceae In : "Bergey's Manual of Systematic Bacteriology, Vol. I", pp. 506-516. The Williams & Wilkins Company, Baltimore, USA.

Gerhardson, B. (2002). Biological substitutes for pesticides. Trends Biotechnol. 20:338-343.

Ghosh, S.B.; L.H.S. Nagi; T.R. Ganapathi; S.M. Paul Khurana and V.A. Bapat (2002). Cloning and sequencing of *Potato virus Y* coat protein gene from an Indian isolate and development of transgenic tobacco for PVY resistance. Current Science 82: 7-10.

Griffin, R.L. (1990). Using the Transmission Electron Microscope in the Biological Sciences, pp. 99-101. Ellis Horwood, New York.

Holt, J.G.; N.R. Krieg; P.H.A. Sneath; J.T. Staley and S.T. Williams (1994). Gram negative aerobic microaerophili rods and cocci. Group 4, In: "Bergey's Manual of Determinative Bacteriology, 9th ed.", pp. 93-153. Williams & Wilkins, Baltimore, USA.

Kloepper, J.W.; R. Rodriguez-Ubana; G.W. Zehnder; J.F. Murphy; E. Sikora and C. Fernandez (1999). Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. Austral. Plant Pathol. 28:21-26.

Koenig, R.C. (1981). Indirect ELISA methods for broad specificity detection of plant viruses. J. Gen. Virol. 55: 53-62.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227: 280-285.

Le-Docte, A. (1927). Commercial determination of sugar beet root using the Sachr Le-Docta process. Int. Sugar J. 29: 488-492.

Leilah, A.A.; M.A. Badawi; E.M. Said; M.H. Ghonema and M.A.E. Abdou (2005). Effect of planting dates, plant population and nitrogen fertilization on sugar beet productivity under the newly reclaimed sandy soils in Egypt. Sci. J. of King Faisal Univ. 6: 1426.

Lot, H.; J. Marrou; J.B. Quiot and C.H. Esvan (1972). Contribution à l'étude du virus de la mosaïque du concombre (CMV). II. Méthode rapide de purification du virus. Ann. de Phytopathologie 4: 25-38.

Madhubala, R.; V. Bhadramurthy; A.I. Bhat; P.S. Hareesh; S.T. Retheesh and R.S. Bhai (2005). Occurrence of *Cucumber mosaic virus* on vanilla (*Vanilla planifolia* Andrews) in India. J. Biosci. 30: 339-350.

Maurhofer, M.; C. Hase; P. Meuwly; J.P. Metraux and G. Defago (1994). Induction of systemic resistance of tobacco to *Tobacco necrosis virus* by the root-colonizing *Pseudomonas fluorescens* strain CHAO: Influence of gac A gene and of pyoverdine production. Phytopathology 84: 139-146.

Minafera, A. and A. Hadidi (1994). Sensitive detection of *Grapevine virus A*, *B* or leafroll associated III from viruliferous mealybugs and infected tissues by cDNA amplification. J. Virol. Methods 47:175-188.

Murphy, J.F.; M.S. Reddy; C.M. Ryu; J.W. Kloepper and R. Li (2003). Rhizobacteria-mediated growth promotion of tomato leads to protection against *Cucumber mosaic virus*. Phytopathology 93:1301-1307.

Palukaitis P.; M.J. Roossinck; R.G. Dietzgen and R.I.B. Francki (1992). *Cucumber mosaic virus*. Adv. Virus Res. 41: 281-348.

Roossinck, M.J. (1999). Cucumoviruses (Bromoviridae)-general features, In: "Encyclopedia of Virology, 2nd edition", pp. 315-320, (Granoof L. & Webster R.G. eds). Academic Press, SanDiego, USA.

Ryu, C.M.; J.F. Murphy; K.S. Mysore and J.W. Kloepper (2004). Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber mosaic virus* by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. Plant J. 39: 381.

Sarma, Y.R.; G. Kiranmani; P. Sreenivasulu; M. Anandaraj; M. Hema; M. Venkatramana; A.K. Murthy and D.V.R. Reddy (2001). Partial characterization and identification of a virus associated with stunt disease of black pepper (*Piper nigrum*) in South India. Curr. Sci. 80: 459-462.

Shehata, Sawsan F. and A.M. El-Borollosy (2008). Induction of resistance against *Zucchini Yellow Mosaic Potyvirus* and growth enhancement of squash plants using some plant growth–promoting rhizobacteria. Australian J. of Basic and App. Sci. 2: 174-182.

Shukla, D.D. and C.W. Ward (1988). Amino acid sequence homology of coat protein as a basis for identification and classification of the potyvirus group. J. of Gen. Virol. 69: 2703-2710.

Stevens, M. (2007). Impact of *Beet mosaic virus*: Is it really a problem?. British Sugar Beet Review 75: 10-12.

Verma, N.; B.K. Mahinghara; R. Ram and A.A. Zaidi (2006). Coat protein sequence shows that *Cucumber mosaic virus* isolate from geraniums (*Pelargonium* spp.) belongs to subgroup II. J. Biosci. 31: 47-54.

Wieslander, L. (1979). A simple method to recover high molecular weight RNA and DNA after electrophoresis separation in low gelling temperature agarose gels. Anal. Biochem. 98: 305.

Xi, D.; L. Lan; J. Wang; W. Xu; B. Xiang and H. Lin (2006). Variation analysis of two *Cucumber mosaic viruses* and their associated satellite RNAs from sugar beet in China. Virus Genes 33: 293-298.

التوصيف الجزيئي لعزلة من بنجر السكر لفيروس موزيك الخيار و مقاومتها بواسطة بعض بكتريا الجذور المحفزة للنمو

منى منصور عرابي، علي محمد البرلسي و ممدوح حسين عبدالغفار

قسم الميكروبيولوجيا الزراعية، كلية الزراعة، جامعة عين شمس، حدائق شبرا ١١٢٤١
ص.ب. ٦٨، القاهره - مصر

خلال هذه الدراسة تم عزل فيروس موزيك الخيار CMV من نباتات بنجر السكر *Beta vulgaris* صنف Gazal و التي كانت تظهر اعراض شبيهة بالاصابة الفيروسية. عزل الفيروس على اساس استخدام الاجسام المضادة عديدة النسل polyclonal و نباتات الزربيح *Chenopodium quinoa* كعائل يعطي بقعا محلية. عزلت ثلاثة من بكتريا الجذور المشجعة للنمو plant growth-promoting rhizobacteria من محيط جذور rhizosphere نباتات البنجر و عرفت مورفولوجيا و فسيولوجيا لتكون *Azotobacter chroococcum*, *Serratia marcescens* and *Pseudomonas fluorescens*. نقى الفيروس من 200 جم اوراق دخان *Nicotiana tabacum* cv. White Burley مصابة حيث اعطت التنقية نسبة $A_{260/280}$ تساوي 1.17 و محصول للفيروس 1.8 مجم. اظهرت صور الميكروسكوب الاليكتروني جزيئات فيروسية كروية بقطر 30 نانوميتر. تم تقدير وزن الغطاء البروتيني للفيروس هدف الدراسة باستخدام اختبار الهجرة الكهربية في جيل البولي اكرالاميد sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) حيث ظهر البروتين خلال جيل التحليل resolving gel في صورة حزمة بوزن 25 كيلودالتون. باستخدام تفاعل البلمرة المتسلسل و النسخ العكسي بعد ربط الجزيئات الفيروسية بالاجسام المضادة المتخصصة immunocapture-reverse transcriptase-polymerase chain reaction (IC-RT-PCR) تمت مضاعفة جين الغطاء الفيروسي و الذي اعطى النتيجة المتوقعة للجين و المتمثلة في ظهور حزم في جيل الاجاروز بوزن 657 bp. تم التاكد من انتماء الفيروس للنوع السيرولوجي الاول serotype 1 عن طريق دراسة التتابع النيكليوتيدي لجين الغطاء البروتيني و مقارنته بالتتابعات الخاصة بخمس عزلات دولية. عوملت نباتات البنجر بالري سواء بالمزارع البكتيرية الخام crude او بالسائل الطافي supernatant الخاص بتلك المزارع، اما بشكل منفرد او على هيئة خليط 1:1 او 1:1:1 v:v:v. خلال المعاملة حقنت النباتات بفيروس CMV هدف الدراسة و بعد مرور شهرين درست النباتات لدرجة الاعراض ووجود الفيروس، و كذلك للوزن الجاف الكلي و محتوى الجذور من السكر. افضل النتائج تم الحصول عليها من خليط 1:1:1 من المزارع البكتيرية الخام حيث وجد ان عشرة نباتات من واقع ثلاثين لم تظهر اعراض اصابه، و قد تم التاكد من خلو ثلاثة من هذه النباتات من الفيروس باستخدام اختبارات الاليزا الغير مباشرة indirect enzyme-linked immunosorbant assay (I-ELISA) و IC-RT-PCR. هذه النباتات اظهرت اعلى مقاييس للوزن الجاف للنبات (90.10 جم) و كذلك محتوى السكر للجذر (30 جم).