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Controlling Soft Rot Disease of Potatoes Caused by *Enterobacter cloacae* sub sp. *dissolvens via* Virulent Bacteriophages

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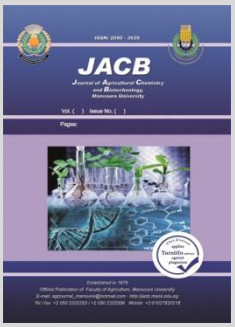

ABSTRACT

Enterobacter cloaca causes bacterial soft rot disease of potatoes**.** Bacteriophages can be used as a biocontrol agent against pathogenic bacteria. The possibility of using lytic phage isolates to control soft rot disease of potato was studied.Isolation and identification of different virulent bacteriophages specific to *Enterobacter cloacae* sub sp. *dissolvens* was took place by using biological and molecular techniques. The efficiency of lytic phage isolates on *E. cloacae* sub sp. *dissolvens* as a biocontrol agent under greenhouse condition was evaluated.The two virulent phage isolates specific to *E. cloacae* sub sp. *dissolvens* were isolated, identified and designated as EdIv-1 and EdIv-2. Mixture of the two virulent phages (EdIv-1 and EdIv-2) could be used as a bio control agent against *E. cloacae* sub sp. *dissolvens* in soil. The data indicated that the number of bacterium in the soil treated with this treatment as a cocktail of phages were considerably lower than in the soil treated with bacterium only in all the different periods and other treatments. In addition the highest percentage of decreased plants was recorded after 30 days (71%). Virulent phage isolates were found to be stable, infecting *E. cloacae* sub sp. *dissolvens.* It could be concluded that cocktail of phages can be used as effective biological control agent against the plant pathogen under the environmental condition in Egypt.

Keywords*: Enterobacter*, Virulent phage, Stability, Biocontrol.

INTRODUCTION

In Egypt, potato has an important position among all vegetable crops, and is used for human consumption, animal feed as well as a source of carbohydrates, alcohol and protein, exporting and processing (Hassan, 2017). One of the most destructive diseases of potatoes and vegetables is soft rot (Kucharek and Bartz, 2000) and it occurs worldwide in many crops (Villavicencio, *et al*. 2011).

Potatoes are susceptible to several bacterial diseases, *e.g*. soft rot caused by bacteria belong to Family Enterobacteriaceae (Carstens, *et al.* 2018). Soft rots affect vegetables such as potatoes, carrot, tomatoes, cucurbits and cruciferous crops (Lunt, 2013). Bacterial soft rot is a leading cause of postharvest losses of potatoes, tomatoes, peppers, lettuce and other fresh fruits and vegetables. The disease can be found on crops in the field, transit, storage and during marketing causing high economic losses (Wells and Butterfield, 1997).

Different lytic *Enterobacter aerogenes* bacteriophages were isolated and purified from sewage water and greenhouse soil by Takikawa, *et al.* (2002) and Zaika, *et al.* (2011) .

RAPD-PCR was used for quick typing and studying the detection and genetic diversity of phage isolates from different bacteria (Kumari, *et al*. 2010 and Gutierrez, *et al*. 2001) as well as *Pectobacterium carotovorum* (Parent, *et al*. 1996) and *Klebsiella pneumonia* (Kumari, *et al*. 2010).

SDS-PAGE technique was used to study the Enterobacter phages and *Serratia marcescens* (Loessner, *et al*. 1993 and Matsushiro, *et al*. 1999).

Little attention was paid to the use of phages to control soft rot and blackleg bacteria in potato. However, Ravensdale, *et al*. (2007) had succeeded in controlling up to 50% soft rot incidence on calla lily tubers inoculated with *Pectobacterium* in greenhouse trials. Phage biocontrol possesses advantages over chemical controls in that tailor-made phage cocktails can be adapted to target specific disease-causing bacteria. Unlike chemical control measures, phage mixtures can be easily adapted for bacterial resistance which may develop over time (Buttimer, *et al*. 2017).

In Egypt, tested 9 phages were effective in controlling bacterial soft rot on potato tubers comparing with the control treatment. In addition, the mixture of these isolates was more efficient in decreasing the incidence and severity of the disease as soft rot from 2 to 4 days after the infestation with *Pectobacterium carotovorum* sub sp. *carotovorum* isolates than the efficient of each of them alone (Hassan, 2017).

This work aimed to study the different control treatments of bacteria causing soft rot disease of potato using cocktail lytic phage isolates. Therefore, isolation and characterization of lytic phages from soil cultivated with potato tubers infecting with soft rot bacteria were performed and important.

MATERIALS AND METHODS

Source of soft rot bacterial isolates and strain

Erwinia carotovora sub sp. *carotovara* ATCC 1063, *Serratia marcescens* sub sp. *marcescens* and *Entrobacter cloacae* sub sp. *dissolvens* as a soft rot bacteria were

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obtained previously by Embaby *et al*. (2017).According to him, all isolates and strains were tested for lysogeny using U.V. irradiation (240 nm).

Source of lytic phages

Sixty samples of soil cultivated with Spunta cultivar of potato tubers were collected from five governorates (*i.e.* Qalyubiyah, Menoufia, Gharbia, Dakahlia and El-Behera) (Ministry of Agric. and Land Reclamation Affairs Sector, A.R.E. 2015). All samples were taken about 15 cm. away from the plant by oak field soil sampling tube and kept at 4ºC after collection for detection of phages.

Detection and isolation of the lytic phages

The previously collected soil samples were used to detect the lytic phages against soft rot bacterial isolates and strains using spot test and plaque assay techniques according to (Othman, 1997). Lytic phages were isolated and purified using the single plaque isolation method (SPI) as described by Mercer and Mills (1960).

Propagation of lytic phages

The liquid culture method was used for propagation of lytic phages and preparing phage lysates as described by Sambrook, *et al*. (1989). Titer of the prepared phage suspensions was determined according to Kiraly, *et al.* (1970).

Characterization of lytic phage isolates

a- Host range pattern

The spot test was used to determine the host range of lytic phage as described by Goodridge, *et al*. (2003).

b- Stability of lytic phage isolates

The thermal inactivation point (TIP), the dilution end point (DEP) and the longevity *in Vitro* (LIV) of lytic phage isolates were determined as described by Akindolire and Ateba, (2019) and Ackermann, (2003). The pH (4 to 12) stability of lytic phage isolates was determined as according to Jamalludeen, *et al*. (2007).

c- Morphological properties of lytic phage isolates

The lytic phages were purified, precipitated and resuspended as described by Embaby, *et al.* (2017). These phages were negatively stained with 2% (W/V) phosphotungstic acid (pH 6.8) according to (Ackermann, 2007). The grids were examined by Jeol-Jem 1010 transmission electron microscope (Electron microscope unit, El – Azhar Univ., Cairo – Egypt).

d- Protein pattern of lytic phage isolates

Protein of lytic purified phage isolates was fractioned by SDS-PAGE as described by Laemmli, (1970).

e- Molecular characters of lytic phage isolates

1- Viral DNA extraction

DNA of lytic phage isolates was extracted as described by Dellaporta, *et al*. (1983). Purity and concentration of lytic phages DNA were determined by the absorbance at 260 and 280 nm using (Nanodrop 2000 C, principal central lab, Fac. of Agric., Cairo Univ.).

2- RAPD-PCR of lytic phages DNA

RAPD-PCR of the lytic phages was done by using RAPD- DNA primers. Eight random DNA oligonucleotide primers were used in the PCR reaction Table (1&2) according to (Roehrdanz and Flanders, 1993). PCR was performed in 25 µl volume tubes, that contained of (2 x) Dream taq Green PCR Master Mix (12.5 µl) ,Primers (2 µl) , Template DNA (50 ng/1 µl) and Nuclease free water (9.5 µl).

Table 1. Nucleotide sequences of the used primers matched with the lytic phages.

Name of primers	Sequences (5' ……. 3')
OPP-13	GGAGTGCCTC
OPN-08	ACCTCAGCTC
OPB-12	CCTTGACGCA
OPH-05	AGTCGTCCCC
OPP-08	GGAGCCCAG
OPS-13	TTCCCCCGCT

Table 2. Nucleotide sequences of the used primers non-matched with the lytic phages.

Name of primers	Sequences (5' ……. 3')
OPC–01	TTCGAGCCAG
OPS–61	TTCGAGCCAG

The DNA amplification programme was performed and bands of PCR product were detected as described by Williams, *et al*. (1996).

The DNA bands were compared with the DNA marker (from 100 to 1000 bp.) and gels were analyzed in Biotechnology lab, Faculty of Agriculture, Cairo University, Egypt.

Biological control of *Enterobacter cloacae* sub sp. *dissolvens* by specific lytic phages cocktail

Under greenhouse condition, a pot experiment was carried out to study the efficiency of the virulent phages cocktail against *Enterobacter cloacae* sub sp. *dissolvens* as a pathogen causing soft rot disease of potato .

Thirty plastic pots sterilized by 70% alcohol were filled with sterilized clay soil 1 Kg soil / pot. One potato tuber of cultivar Spunta potato tubers was cultivated in each pot after sterilization as described by Delfan, *et al*. (2014). All tubers of Spunta cultivar were cut into halves and cultivated in the pots and irrigated with tap water up to 3 – 4 cm above of the soil surface. When potato plants were 7 days old, the pots were divided into five groups, each group comprised 6 pots. The groups were subjected to the different treatments as below:

Treat. 1: Inoculation with liquid culture (10^8 cfu/ml) of *Entrobacter cloacae* sub sp. *dissolvens* (5 ml/pot).

Treat. 2: Inoculation with lytic EdIv-1 phage suspension (10^8 pfu/ml) (5ml phage suspension/pot).

Treat. 3: Inoculation with liquid culture *Entrobacter cloacae* sub sp. *dissolvens* (5 ml/pot) and lytic EdIv-1 phage suspension (5 ml/pot).

Treat. 4: Inoculation with liquid culture of *Entrobacter cloacae* sub sp. *dissolvens* (5 ml/pot) and 5 ml of phage suspension of each lytic EdIv-1 and EdIv-2 phages (10^8 pfu/ml).

Treat. 5: Un-inoculated pots (control).

Data recorded

Pots of all treatments were irrigated with tap water and the following determinations were recorded.

Total count of *Enterobacter cloacae* sub sp. *dissolvens* was estimated in pots of each treatments after 15, 30, 45 and 60 days using serial decimal dilution as described by Marei, *et al*. (2018).

Also, the plant growth parameters, *i.e.* plant height (cm) and number of leaves plant were determined every 15 days upto 60 in the different treatments.

Statistical analysis

Data were statistical analyzed according to (Duncan, 1955).

RESULTS AND DISCUSSION

Detection and isolation of lytic phages

Two lytic phages specific to *Enterobacter cloacae* sub sp. *dissolvens* were detected in the soil samples collected from Menoufia Governorate by spot test and isolated by single plaque isolation (SPI) technique. Two various types of plaques diameter were produced by the isolated phages. The 1st plaque type had 2 mm and the 2nd plaque had 3 mm diameter and both were clear area with center, circular plaque and opaque halos in their margins (Fig. 1) . These two lytic phages were designated as EdIv-1 and EdIv-2 respectively. Similar result was recorded by Takikawa, *et al.* (2002) .

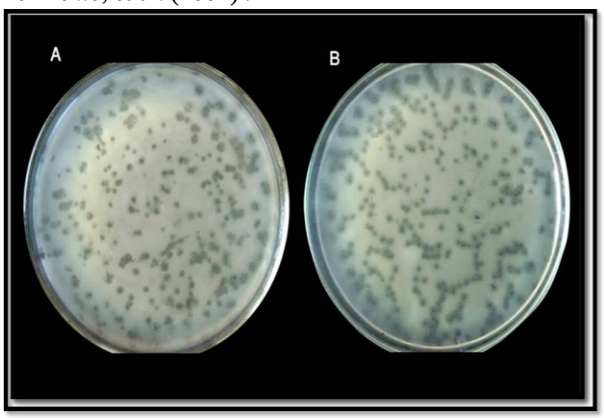

Figure 1. Single plaques of EdIv-1 (A) and EdIv-2 (B) virulent phages specific to *Enterobacter cloacae* sub sp. *dissolvens*.

Characterization of lytic phage isolates

Host range pattern of EdIv-1 and EdIv-2 phages

The lytic phage EdItv-1 was infectious to *Entrobacter cloacae* sub sp. *dissolvens* and *Erwinia carotovora* sub sp. *carotovara* ATCC 1063 among the three bacterial isolates tested. Moreover, the lytic phage (EdItv-2) was found to be infectious only to *Entrobacter cloacae* sub sp. *dissolvens* Table (3). Similar results were obtained by (Barnet, 1972).

Table 3. Host range of EdIv-1 and EdIv-2 phages.

Soft root bacterial isolates	lytic phage (EdItv-1)	lytic phage (EdItv-2)
Serratia marcescens sub sp. *marcescens*	-	-
Entrobacter cloacae sub sp. *dissolvens*	+	+
Erwinia carotovora sub sp. *carotovara* ATCC 1063	+	-

+ = Lysis. - = No lysis.

Stability of EdIv-1 and EdIv-2 phages

Data in Tables (4) show that EdIv-1 phage isolate was active till 74ºC and lost its infectivity at 76ºC while EdIv-2 phage isolate was active till 68ºC and lost its infectivity at 70ºC. *i.e.* the thermal inactivation point was found to be 76ºC and 70ºC for EdIv-1 and EdIv-2 phage isolates, respectively. The DEP of EdIv-1 and EdIv-2 phage isolates were 10^{-5} for both phages. The LIV *in vitro* of EdIv-1 phage was active till 6 days and EdIv-2 phage was active till 5 days of storage at room temperature. The lytic phages were found to be stable to wide range of pH 5 to 9 for EdIv-1 phage and pH 5 to11 for EdIv-2 phage after incubation at 37ºC for 24hrs. These results are in agreement with (Ritchie and Klos, 1979) for phages infected *Erwinia amylovora* PEal (h), PEal(nh) and PEa7 phages (Lim, *et al.* 2013 and Marei, *et al.* 2017).,for bacteriophage of *Pectobacterium carotovorum* subsp. *carotovorum.*

Table 4. Stability properties of EdIv-1 and EdIv-2 phages.

Bacteriophages	Stability of lytic phage isolates			
	TIP	DEP	LIV	pH
EdIv-1	74ºC	10^{-5}	6 days	5 to 9
EdIv-2	68ºC	10^{-5}	5 days	5 to11

Morphological properties of EdIv-1 and EdIv-2 phages

As shown in Figure (2), the EdIv-1 and EdIv-2 phages were found to have hexagonal icosahedral heads of 50 and 75 nm in diameter and contractile tails of 150 and 175 nm in length, respectively. On the basis of the particle morphology of both phages, they seem to belonging to family Myoviridae. The obtained results are in agreement with those obtained by Hassan, (2017) and Marei, *et al.* (2017) for phage of *Pectobacterium carotovorum* subsp. c*arotovorum*.

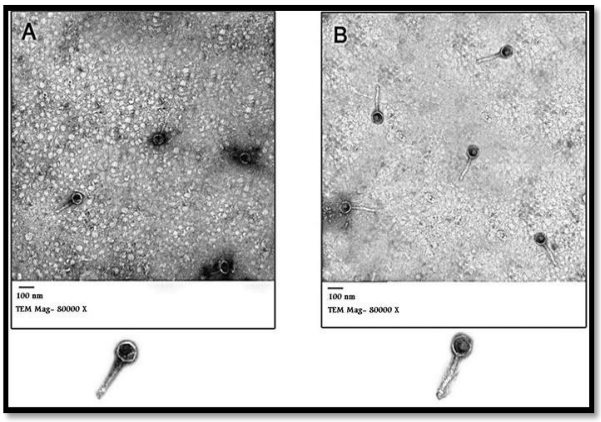

Figure 2. Electron micrographs of the EdIv-1 (A) and EdIv-2 (B) virulent phage isolates negatively stained with (2%) phosphotungstic acid at pH 6.8.

Protein pattern of EdIv-1 and EdIv-2 phages

As shown in Figure (3), the lytic phage isolate (EdIv-1) have 6 bands with molecular weights of 128, 97, 68, 50, 28 and 16 KDa. Whereas, the lytic phage isolate (EdIv-2) have 11 bands with molecular weights of 132, 73, 68, 52, 50, 48, 33, 30, 23, 21 and 20 KDa. when compared with marker protein. These results are in agreement with those obtained by Elshayeb, *et al.* (2011) who found that the protein profiles of *E. coli* bacteriophage showed three major bands with molecular weight of 47, 35 and 16 KDa. and Kharina, *et al.* (2012) for *Serratia marcescens* IMBG291 phage proteins were observed six major protein bands 86.6, 45, 41.9, 35, 30.6 and 21.4 KDa.

Molecular characters of EdIv-1 and EdIv-2 phages

Purity and concentration of viral DNA

The purity and concentration of DNA isolated from EdIv-1 and EdIv-2 lytic phage isolates were determined by the absorbance at 260 and 280 nm. The DNA concentrations of EdIv-1 and EdIv-2 lytic phages were 37.6 and 169.7 ng/µl, respectively. The ratio of A_{260}/A_{280} and A_{280}/A_{260} as a measure of DNA purity were found to be 1.45, 1.41, and 0.69, 0.71 for EdIv-1 and EdIv-2 lytic phages isolates, respectively. The lytic

phage isolates EdIv-1 and EdIv-2 contained ds-DNA as a viral genome. These results are in agreement with those obtained by Gutierrez, *et al*. (2011).

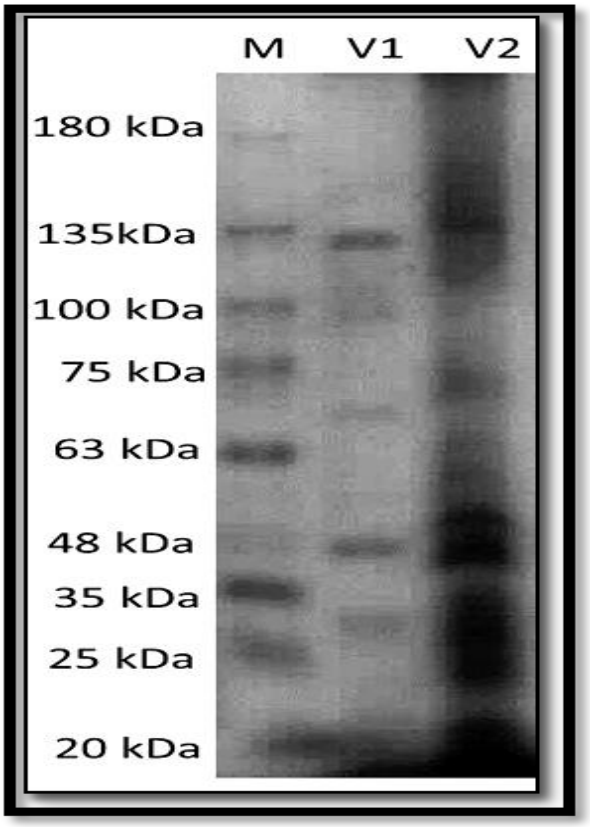

Figure 3. SDS-PAGE (14%) electrophoresis of protein of the purified virulent phage isolates EdIv-1 lane (2) , EdIv-2 lane (3) and marker protein lane (1).

RAPD-PCR of EdIv-1 and EdIv-2 Phages

The RAPD-PCR was used to study the genetic diversity of DNA products of the EdIv-1and EdIv-2 phages. Only six primers succeeded to generate reproducible polymorphic DNA products with different size with each primer. The total amplified products were 63 DNA bands, generated by OPP-13, OPN-8, OPB-12, OPP-8, OPH-5 and OPS-13 primers.

Forty six bands were found to be unique polymorphic bands for EdIv-1 and EdIv-2 phage isolates Figure (4) illustrated with Table (5) , and 17 bands were found to be monomorphic with both phages. The sizes of the amplified fragments were ranged from 160 to 2000 bp.

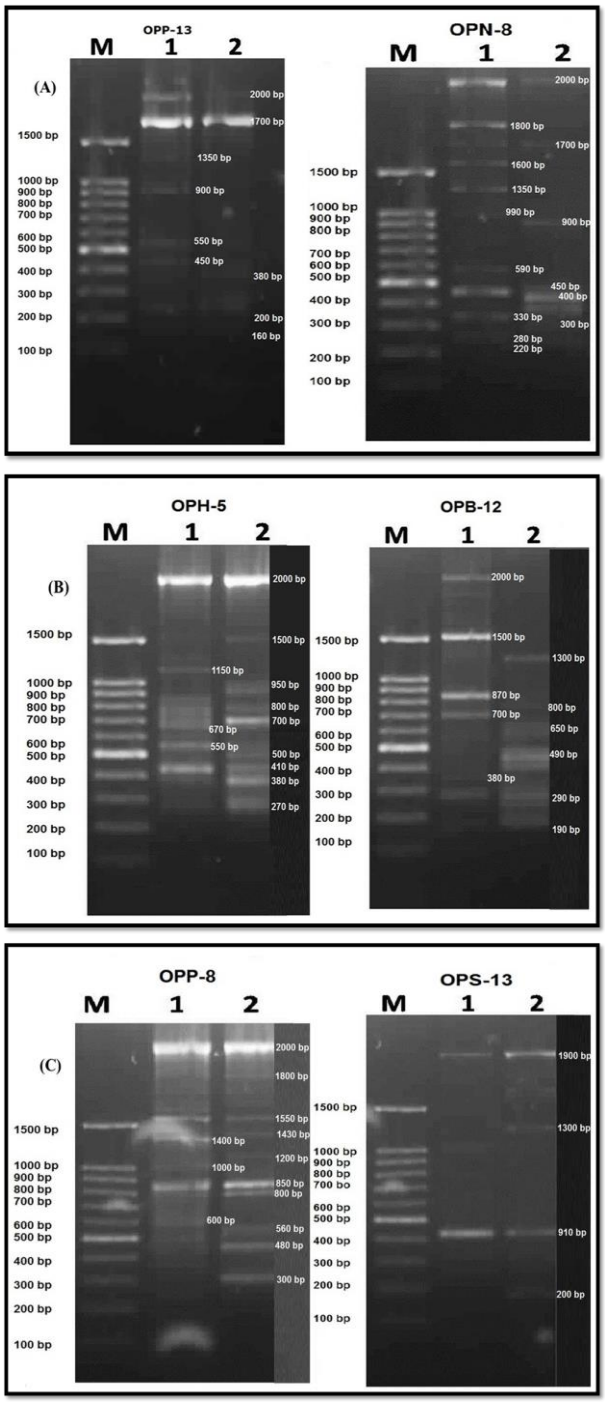

Figure 4. RAPD patterns with primers for virulent phages (A: OPP – 13, OPN-8) (B: OPH – 5, OPB-12) and (C: OPP – 8, OPS-13). (Lane M: DNA ladder, Lane 1: phage EdIv-1 and lane 2: phage EdIv-2).

Table 5. The unique polymorphic amplified DNA bands and polymorphism percentage for each primer for EdIv-1and EdIv-2 phages.

Primers	Molecular size range (bp.)	No. of bands			Polymorphism %
		Monomorphic	Polymorphic	Total	
OPP – 13	160-2000	4	5	9	55.55
OPN – 8	220-2000	3	11	14	78.57
OPB – 12	190-2000	1	10	11	90.91
OPP – 8	300-2000	4	9	13	69.23
OPH – 5	270-2000	3	9	12	75.00
OPS – 13	200-1900	2	2	4	50.00
Total		17	46	63	69.89
Average		2.83	7.67	10.5	--

For OPP – 13 primer, 5 DNA polymorphic bands were appeared and 4 DNA monomorphic bands were appeared with EdIv-1 and EdIv-2 phages. The sizes ranged from 160-2000 bp. According to OPN – 8 primer, 11 DNA polymorphic bands showed and 3 DNA monomorphic bands were showed with EdIv-1 and EdIv-2 phages. The range in size bands was found between 220 to 2000 bp. Regarding the OPB – 12 primer, ten polymorphic bands and one DNA monomorphic band were recorded with EdIv-1 and EdIv-2 phages. The sizes ranged from 190 to 2000 bp. Moreover, primers OPH – 5 generated 9 polymorphic bands and 3DNA monomorphic bands were showed with EdIv-1 and EdIv-2 phages. The size of the bands generated ranged between 270 - 2000 bp. For OPP – 8 primer, 9 DNA polymorphic bands and 4DNA monomorphic bands were appeared with EdIv-1 and EdIv-2phages. The sizes ranged from 300-2000 bp. According to OPS –13 primer, 2 DNA polymorphic bands showed and 2 DNA monomorphic bands were showed with EdIv-1 and EdIv-2 phages. The range in size of the bands was found between 200 to 1900 bp. These results agreement with those obtained by Gutierrez, *et al.* (2011), Denyes, *et al*. (2014) and Czajkowski, *et al*. (2015) .

Biological control of *Enterobacter cloacae* sub sp. *dissolvens* by specific lytic phages cocktail

Count of bacteria in soil

As shown in Table (6) and Figure (5) it was found that the number of bacterium *Enterobacter cloacae* sub sp. *dissolvens* in the soil cultivated with potato plants under greenhouse experiment and inoculated with different treatments (bacterium + EdIv-1 phage) and (bacterium + EdIv-1 phage and EdIv-2phage as a phages cocktail) were considerably lower than the treatment with the bacterium only at all experimental periods (15 to 60 days). In addition the highest reduction was found after 30 days (71%) followed by 45 days (58%) when both phages were applied. These results are in agreement with those obtained by Lim, *et al*. (2013), Hassan, (2017) and Carstens, *et al*. (2018) when, used different bacteriophage isolates as a biocontrol agents against soft rot bacteria.

Table 6 . Number of *Enterobacter cloacae* sub sp. *dissolvens* (cfu / ml) in the soil cultivated with potato plants inoculated with different treatments.

Treatments	Zero time	15 days	30 days	45 days	60 days
Control	0 x 10^6	37 x 10^6	56 x 10^6	85 x 10^6	55 x 10^6
Bacterium only	35 x 10^6	71 x 10^6	95 x 10^6	117 x 10^6	87 x 10^6
Only EdIv-1 phage	0 x 10^6	51 x 10^6	42 x 10^6	59 x 10^6	66 x 10^6
Bacterium+ EdIv-1 phage	35 x 10^6	47 x 10^6	37 x 10^6	43 x 10^6	77 x 10^6
Bacterium + EdIv-1 and EdIv-2 phages	35 x 10^6	51 x 10^6	27 x 10^6	49 x 10^6	67 x 10^6

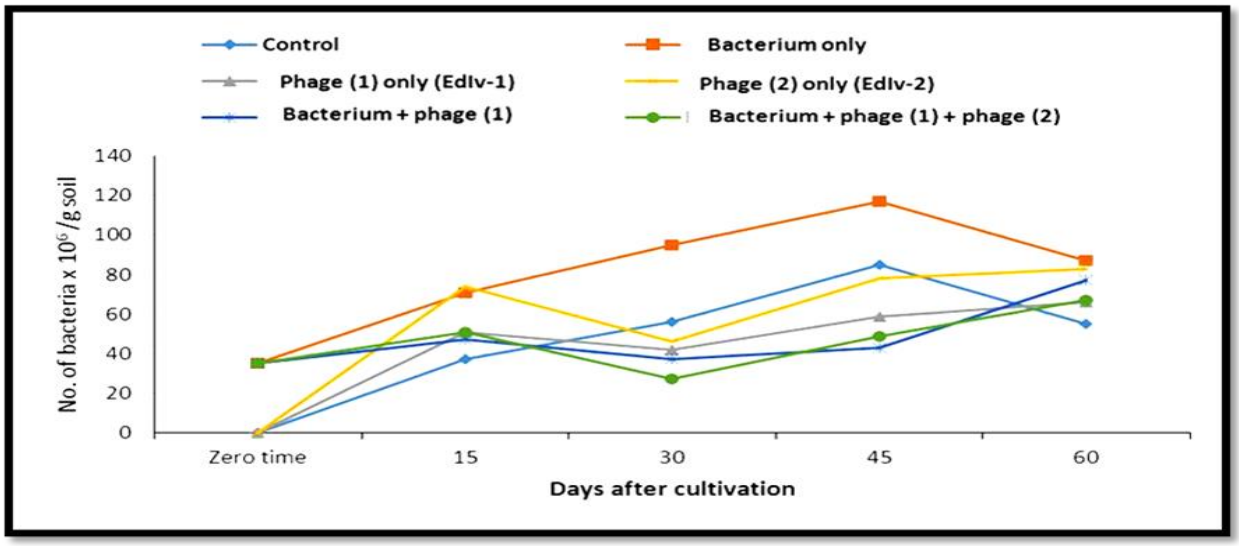

Figure 5. Density of *Enterobacter cloacae* sub sp. *dissolvens* (cfu/ml) in the soil cultivated with potato plants inoculated with different treatments during the different growth stages.

Plant height and number of leaves

Data presented in Table (7) indicate that significant increases in plant height and number of leaves/plant were recorded in plants inoculated with + EdIv-1 phage and bacterium + EdIv-1 and EdIv-2 as a phages cocktail, as compared to those in other treatments (*i.e.* control, bacterium only and only EdIv-1 Phage). These increases were recorded at all experimental periods. These results are in agreement with those obtained by Lim, *et al.* (2013), Hassan, (2017) and Carstens, *et al.* (2018) when, used different bacteriophage isolates as a biocontrol agents against soft rot bacteria.

Table 7. Effect of different viral treatments on growth parameters of inoculated potato plants.

Treatments	Plant height /cm	No. of leaves
Control	60.33 $^{a\,b\,c}$	10.33 ^{bc}
Bacterium only	55.06 ^{c}	7.78 ^{d}
Only EdIv-1 phage	65.89 ^{a}	11.17 ^{b}
Bacterium + EdIv-1 phage	66.5 ^{a}	12.05 ^{a}
Bacterium + EdIv-1 + EdIv-2 phages	63.72 ^{ab}	12.83 ^{a}
LSD at 5 %	8.4145	1.3475

***The same letters are not significantly different.**

CONCLUSION

Generally, on the basis of the obtained results it can be concluded that, EdIv-1, EdIv-2 virulent phage isolates were stable under different conditions. Since, these two lytic phages (as a cocktail) were successfully used as a bio-control agent against *Enterobacter cloacae* sub sp. *dissolvens*. The use of lytic phages specific to *Enterobacter cloacae* sub sp. *dissolvens* as biocontrol agent is highly recommended to control soft rot disease of potatoes under Egyptian conditions.

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السيطرة على مرض التعفن الطرى فى البطاطس الناجم عن *Enterobacter cloacae* sub sp. *dissolvens* بواسطة الفاجات العدوانية

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تتواجد البكتيريا المعوية *Enterobacter cloacae* بشكل شائع بالعديد من النباتات والحيوانات والتربة والمياه باعتبارها بكتريا ملوثًة لهم. ولوحظ أن هذه البكتريا تسبب مرض التعفن الطرى البكتيرى فى البطاطس. وأمكن أستخدام البكتريوفاجات كمكافح حيوى ضد هذه البكتيريا المرضية. لذلك يهدف هذا العمل إلى دراسة إمكانية أستخدام عزلات الفاج المعزولة كمكافح حيوى للسيطرة على مرض التعفن الطري للبطاطس.وأعتمدت الطرق والمواد المستخدمة لتحقيق هدف هذه الدراسة على عزل وتعريف فاجات عدوانية مختلفة متخصصة فى إصابة بكتيريا *Enterobacter cloacae* sub sp. *dissolvens* وذلك إعتمادا على أستخدام التكنيكات البيولوجية والجزيئية لذلك. وأيضا تم دراسة كفاءة عزلات الفاجات العدوانية المختلفة كمكافح حيوى ضد بكتيريا *Enterobacter cloacae* sub sp. *dissolvens* تحت ظروف الصوبة الزجاجية.وقد تم عزل أثنان من عزلات الفاجات العدوانية المتخصصة فى الأصابة لميكروب *Enterobacter cloacae* sub sp. *dissolvens* ، وتعريفهما وتسميتها ب EdIv-1 و EdIv-2. كما أمكن أستخدام خليط من هذه الفاجات العدوانية المعزولة المختلفة معا (EdIv-1 و EdIv-2) كمكافح حيوي ضد ميكروب *Enterobacter cloacae* sub sp. *dissolvens* في التربة تحت ظروف الصوبة الزجاجية. وأوضحت النتائج وجود أنخفاض ملحوظ فى عدد البكتيريا في التربة التي عوملت بهذه المعاملة كمزيج من الفاجات العدوانية وكان أقل بكثير مما هوعليه في التربة المعاملة بالبكتيريا فقط في جميع الفترات المختلفة خلال مدة التجربة وكذلك مقارنة مع المعاملات الأخرى بالتجربة. بالإضافة إلى ذلك كما سجلت أعلى نسبة (71٪) فى أرتفاع طول النباتات المعاملة بهذا المزيج بعد 30 يومًا من المعاملة.وترجع الأهمية التطبيقية لهذه الدراسة الى أمكانية أستخدام مزيج من الفاجات العدوانية المعزولة المتخصصة كمكافح حيوى ضد مسببات الأمراض النباتية تحت الظروف البيئية.

الكلمات المفتاحية : البكتيريا المعوية ، البكتريوفاج ، الثبات ، المكافحة الحيوية.