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ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES SPECIFIC TO *AZOSPIRILLUM* SP.

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ABSTRACT

Nine bacteriophages specific to *Azospirillum*sp. were successfully isolated from the rhizosphere soil of wheat plants (*Triticumaesativum*), growing in the Experimental Farm at Faculty of Agriculture, Minia University, Minia, Egypt. Different characteristics of the phage isolates (*i.e.* optimum pH for infection, thermal inactivation point, sensitivity to U.V. radiation, longevity *in vitro* and DNA restriction pattern) were studied to characterize and differentiate the phage isolates. On the basis of the differences in the studied characteristics of these phages, the nine phage isolates were divided into three groups (A, B, and C). Phages of each group exhibited the same features. Therefore, the phage isolates of each group were considered one phage type. *i.e.* the nine phage isolates are belonging to three phage types. The three phage types were designated ∅Az1, ∅Az2 and ∅Az3 for phages of group A, B and C, respectively. Each of the phage types was examined by electron microscope. All phage types were found to be of head and tail type. The phage type ∅Az1 was found to be belonging to family *Podoviridae*. Whereas, ∅Az2 and ∅Az3 were found to be belonging to family *Siphoviridae.* One step growth curve experiment was carried out for each phage type. The latent period of each phage type was estimated and was found to be 10 min, 20 min and 15 min and burst size calculated to be 75 pfu/cell, 93 pfu/cell and 82 pfu/cell for ∅Az1, ∅Az2 and ∅Az3, respectively.

Keywords: bacteriophage, *Azospirillum*sp., UV radiation,one-step growth curve.

INTRODUCTION

Plant-beneficial bacteria, including some strains belonging to the genus *Azospirillum*, are usually referred to as plant growth-promoting rhizobacteria. Members of the genus *Azospirillum* represent a group of free-living nitrogen fixing microorganisms that stimulate the growth of cereals and grasses leads to an increase of crop yield up to 30%. The actual benefit due to biological nitrogen fixation has been reported and plant growth promotion by *Azospirillum* spp. seems to be due mainly to production of phytohormones (Bashan *et al.*, 2004 and Dobbelaere *et al.*, 2001).

Bacteriophage or phages for short are viruses that infect only bacteria. Bacteriophages are of widespread occurrence and are usually readily isolated from areas, which contain the appropriate host bacteria. These viruses are of a particular interest, since they are likely to have a significant role in the ecology of their hosts specially those of economically importance in industrial and agricultural purposes. Hammad (1998 and 1999) successfully isolated bacteriophages specific for *Azotobacter, Azospirillum* and *Bacillus megaterium* from rhizosphere soil of different plants.

Many studies described the specific bacteriophages for*Azospirillum* sp. (Franche and Elmerich, 1981; Elmerich*et al.,* 1982; Germida, 1984 and Boyer *et al.*, 2008).

Phages can be characterized by culture lysis test, plaque morphology and particle size and morphology as revealed by direct electron microscopy and host specificity. Previous investigations on bacteriophages have used combination of these features to characterize and differentiate between phage isolates, *e.g.* **(**Elsharouny, 2007; Fathy, 2008 and Farahat, 2016).

This work aimed to study the presence of bacteriophages specific to *Azospirillum spp.* in the rhizosphere soil of wheat plants (*Triticumaestivum*). Moreover, the different characteristics of the isolated phages (*i.e.* optimum pH for infection, thermal inactivation point, sensitivity to U.V. radiation, longevity *in vitro* particle, DNA restriction pattern, size and morphology) were also studied to characterize and differentiate the phage isolates.

MATERIALS AND METHODS

Soil sample: A soil sample was collected from rhizosphere of wheat plants (*Triticumaestivum*) growing in the Experimental Farm at Faculty of Agriculture, Minia University, Minia, Egypt. The collected soil sample was used as a source of *Azospirillum* and bacteriophages.

Bacteria used: *Azospirillum* isolate was isolated from the collected rhizosphere soil sample of wheat (*Triticumaestivum*) as described by **(**Dobereiner*et al*., 1976).

Isolation of Bacteriophages:The liquid enrichment technique of Adams(1966) with minor modification as described by Hammad (1989) was

used to isolate phages of *Azospirillum* from the collected rhizosphere soil sample.

Detection of bacteriophages: The spot test was used for detection of bacteriophages of *Azospirillum* as described by Adams (1966).

Purification of bacteriophage isolates: The single plaque isolation technique was used to obtain pure single phage isolates as described by Kiraly.*et al.*, (1970).

Preparation of high titer phage suspension: Agar double layer plates method described by Maniatis*et al.* (1982) was used to prepare the high titer phage suspension for each single phage isolate as described by Hammad and Dora (1993) andFarahat (2016).

Characterization of bacteriophages:

a) **The optimum pH level:** Nine eppendorf tubes each containing 1 ml SM media with various pHs (*i.e.* 4 upto 12) were prepared. The pH was adjusted with NaOH (0.1 N) and HCl (0.1 N). Individual plaques for each single isolate of phages were transferred to the prepared tubes (plaque/tube). Tubes were incubated at 30°C for 60 min. then 5µl from each tube were spotted over double agar layer plates, containing the appropriate *Azospirillum* indicator bacteria, followed by incubation at 30-33ºC for 24-48 h. Four replicates were employed for each phage isolate. Diameters of the lysed spots were measured. The average values of the replicates were calculated.

b) **Thermal inactivation point:** Nine eppendorf tubes each containing 200 µl of high titer phage suspension of each single phage isolate were prepared. Tubes were heated in water baths adjusted at 50, 55, 60, 65, 70, 75, 80, 85, 90 and 95°C for 10 min, then cooled under tap water. After heat treatment 10µl from each tube was spotted over double agar layer plates containing the *Azospirillum* as indicator bacteria. Plates were inspected for lysed spots after 24-48 h incubation at 30°C.

c) **Sensitivity to ultraviolet irradiation:** Five ml of high titer phage suspension of each single phage isolate were put in a petri dish placed at distance of 20 cm from U.V. lamp of 260 nm wave length. Ten µl of each irradiated phage suspension were spotted over double agar layer plates that containing the appropriate *Azospirillum* indicator bacteria, after 5, 10, 20, 30, 40, …. up to 100 min. exposure to UV irradiation. Plates were inspected for lysed spots after incubation for 24-48 h at 30°C.

d) **L**

ongevity in vitro: Eppendorf tubes each containing 1.5ml of high titer phage suspension were kept at room temperature. Samples were assayed qualitatively every 12 hrs. according to the method of Yoshida *et al.* (2006).

e) **Extraction of bacteriophages DNA:** The DNA of each phage isolate was extracted as described by Maniatis*et al.* (1982) with minor modification (**Compos** *et al*., 2003).

f) Digestion of bacteriophages DNA with restriction enzyme (*Eco*RI): The reactions were assembled by mixing the following components in a sterile 0.5 ml eppendorf vial:

- 10x buffer 2 µl
- Bacteriophage DNA 1µg
- Restriction enzyme (*Eco*RI) 3-5U
- Sterile deionized water upto final volume of 20 µl

The reactions were incubated for two hrs at 37ºC. Five µl of bromophenol blue dye stop [450mM tris borate pH 8.3; 50 mM EDTA; 50% (v/v) glycerol; 0.2% (w/v) bromophenol blue] were added and the reactions were heated at 60ºC for 5 min to stop the reaction. The digested DNAs were stored at 4ºC until ready for analysis by agarose gel electrophoresis.

Agarose gel electrophoresis was carried out using the tris-acetate EDTA buffer (TAE) (40 mM Tris-HCl, 4 mM sodium acetate and 1 mM EDTA pH 7.9). The digested DNA samples were analyzed by horizontal electrophoresis in 1.0% agarose. The gel was stained in 1 µg/ml of ethidium bromide for 30 min. and destained for another 30 min. in about 1 L. of tap water **(Dillon *et al*., 1985)**. The stained gel was examined using an ultraviolet transilluminator with wavelength of 302 nm. The DNA bands which fluoresced orange were photographed using gel documentation system.

g) **Electron microscope examination:** The electron microscope grids were prepared to **examine** each of the isolated phages as described by Hayat and Miller (1990) and stained by 0.5% uranyl acetate pH 4.5 (Stacey *et al*., 1984). The grids were examined at 50 kv in transmission electron microscope (Joel, Model GEM 1010) in Sohag University - Sohag - Egypt.

h) **One-step growth curve:** One-step growth curve experiments were carried out as described by Adams (1966) with minor modification to determine the latent time and phage burst size. Two hundred µl of phage suspension (10^{10} pfu/ml) was mixed with 100 µl of exponential phase culture of *Azospirillum* (10^{8}cfu/ml) and incubated at room temperature for 60 seconds for phage adsorption. The mixture was centrifuged at 12000 rpm for 5 min to remove free phage particles. The pellet was resuspended in 5 ml of SM media, and the culture was continuously incubated at room temperature. Samples were taken at 5 min intervals upto 60 min., and phage titer was determined. Burst sizes of phages were calculated by dividing the phage titers at

plateau phase by the initial phage titers.

RESULTS

Isolation and purification of bacteriophages: Bacteriophages specific to *Azospirillum* sp. were successfully isolated from a soil sample collected from rhizosphere of Wheat (*Triticumaestivum*).The spot test was used for detection of phages in the collected rhizosphere soil sample. As shown in figure (1) the spot test indicates that phages of*Azospirillum* sp. were found to be common in the collected soil sample.

Figure (1): A bacterial lawn of *Azospirillum* sp. spotted with drops of the prepared phage lysate and incubated for 24-30 h. at 30°C. The lysed spots can be clearly seen.

The single plaque isolation technique was used to obtain pure phage isolates of *Azospirillum* sp. As shown in figure (2) the phages specific to *Azospirillum* sp. formed single plaques of different morphologies. Nine single plaques morphologically different were selected and kept as pure phage isolates. The isolated phages formed circular single plaques of 1 to 4 mm in diameter and clear in appearance.

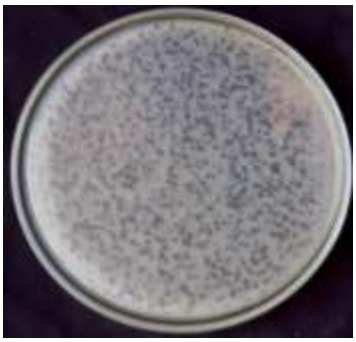

Figure (2): A plate containing single plaques of bacteriophages specific to *Azospirillum* sp. The differences in morphology of the single plaques can be clearly seen.

High titer phage suspension:

One hundred ml of high titre phage suspension was prepared for each phage isolate of *Azospirillum* sp. The titers of the prepared suspensions were found to be ranging from 10^{10} to 10^{12} pfu/ml.

Characteristics of the isolated bacteriophages:

The different characteristics of the nine phage isolates of *Azospirillum* sp. were studied to find out if these phage isolates are different in types or similar.

a- The optimum pH for phage infection:

The infectivity of the nine phage isolates of *Azospirillumspp.*was studied at various pH levels (pH 4 – 12). As shown in Table (1) all phage isolates formed lysed spots at all pH levels up to pH 12. At pH 8 all phage

isolates formed lysed spots wider than those formed at other pH levels tested.

Table (1): Stability of bacteriophages specific to*Azospirillum* sp. to different pH levels.

Phage No.	pH levels								
	4	5	6	7	8	9	10	11	12
	Diameter of the lysed spots (mm.)								
1	10.0	10.5	11.0	11.5	12.0	10.6	10.0	9.0	8.4
2	10.0	10.5	10.9	11.5	12.6	11.8	11.2	9.5	9.0
3	8.0	8.8	9.0	9.5	10.0	9.4	9.0	8.7	8.0
4	10.0	10.8	11.0	11.5	12.0	11.5	11.0	10.0	9.3
5	10.0	10.5	11.0	11.7	13.0	11.5	10.5	10.2	9.6
6	10.0	10.4	10.9	11.4	12.7	12.0	11.5	11.0	10.3
7	9.5	10.0	10.6	11.0	13.0	12.6	12.0	11.4	11.0
8	10.0	10.6	11.5	12.0	13.0	12.0	10.5	10.0	9.5
9	9.5	10.0	11.2	12.5	13.4	12.7	12.0	11.4	10.0

b- Thermal inactivation pointof

The phage isolates: As shown in Table (2) according to the similarity in the thermal inactivation point, bacteriophages of *Azospirillum* were grouped. The nine bacteriophage isolates of *Azospirillum*were divided into three groups (group A, B and C). Each group contained the phage isolates which exhibited the same thermal inactivation point.The less thermal stability for *Azospirillum* bacteriophages was recorded for phages of group B which contains phage isolates No. 4, 5, 8 and 9. They were inactivated at 55°C for 10 min. Whereas, the highest thermal inactivation point (90°C for 10 min.) was recorded for phages of group A which comprised phage isolates No. 1, 3 and 6.

Table (2): Thermal inactivation points of bacteriophages specific to *Azospirillumspp.*exposed to 50 - 95°C for 10 min.

Phage Group	Phage No.	Temperature (C°)									
		50	55	60	65	70	75	80	85	90	95
A	1	+	+	+	+	+	+	+	+	-	-
	3	+	+	+	+	+	+	+	+	-	-
	6	+	+	+	+	+	+	+	+	-	-
B	4	+	-	-	-	-	-	-	-	-	-
	5	+	-	-	-	-	-	-	-	-	-
	8	+	-	-	-	-	-	-	-	-	-
	9	+	-	-	-	-	-	-	-	-	-
C	2	+	+	-	-	-	-	-	-	-	-
	7	+	+	-	-	-	-	-	-	-	-

+ = Lysis - = No lysis

c- Sensitivity to Ultraviolet Irradiation:

Sensitivity of the isolated phages of *Azospirillum* to UV radiation at wave length of 260nm was studied. As shown in Table (3). The UV radiation at wave length of 260 nm inactivated the isolated phages at different exposure time.

Table (3): Effect of U. V. radiation (260 nm) on ten isolates of bacteriophages specific for *Azospirillum*.

Phage Group	Phage No.	Exposure time (min.)											
		5	10	20	30	40	50	60	70	80	90	95	100
A	1	+	+	+	+	+	+	+	+	+	-	-	-
	3	+	+	+	+	+	+	+	+	+	-	-	-
	6	+	+	+	+	+	+	+	+	+	-	-	-
B	4	+	+	+	+	+	-	-	-	-	-	-	-
	5	+	+	+	+	+	-	-	-	-	-	-	-
	8	+	+	+	+	+	-	-	-	-	-	-	-
	9	+	+	+	+	+	-	-	-	-	-	-	-
C	2	+	+	+	+	-	-	-	-	-	-	-	-
	7	+	+	+	+	-	-	-	-	-	-	-	-

+ = Lysis - = No lysis

According to the obtained results in Table (3), the isolated phages were divided in to three groups. Each group comprised number of phage isolates which inactivated after the same exposure time. The most tolerant phage isolates were found in group A that comprised phage isolates No. 1, 3 and 6. Phages of this group lost their infectivity after exposure to UV for 80 min. Group B contained phage isolates No. 4, 5, 8 and 9 that inactivated after exposure for 40 min. group C comprised the most sensitive phage isolates (isolates No. 2 and 7) which inactivated after 30 min.

d- Longevity *in vitro* of the isolated phages:

As shown in Table (4) on the basis of the longevity *in vitro* study the nine phage isolates were classified to three groups (A, B and C). The phage isolates No. 1, 3 and 6 were survived for 132 hours at room temperature. Therefore, these three phages were classified in group (A). Moreover, group (B) comprised phage isolates No. 4, 5, 8 and 9. Each of these four phage isolates were survived for 120 hours at room temperature. Finally, group (C) includes phage isolates No.2 and 7. These two phages of group (C)

survived for 48 hours at room temperature.

Table (4): longevity of the isolated phages stored at room temperature (longevity *in vitro*).

Phage Group	Phage No.	Incubation period at room temperature (hours)											
		12	24	36	48	60	72	84	96	108	120	132	144
A	1	+	+	+	+	+	+	+	+	+	+	+	-
	3	+	+	+	+	+	+	+	+	+	+	+	-
	6	+	+	+	+	+	+	+	+	+	+	+	-
B	4	+	+	+	+	+	+	+	+	+	+	-	-
	5	+	+	+	+	+	+	+	+	+	+	-	-
	8	+	+	+	+	+	+	+	+	+	+	-	-
	9	+	+	+	+	+	+	+	+	+	+	-	-
C	2	+	+	+	+	-	-	-	-	-	-	-	-
	7	+	+	+	+	-	-	-	-	-	-	-	-

+ = Lysis - = No lysis

e- Genome size of the phage isolates:

As shown in Figure (3) and Table (5) *Azospirillum* phage isolates No. 1, 3 and 6 which classified in group (A) on the basis of the optimum pH, thermal stability, sensitivity to U.V. radiation and longevity *in vetro* exhibited identical DNA restriction patterns with EcoRI and were found to contain genomic DNA of the same size (22.570 kbp). Similarly, each *Azospirillum* phage isolate in group B (phage isolates No. 4, 5, 8 and 9) contained genomic DNA of 24.510 kbp and all of these four phages exhibited identical DNA restriction patterns with EcoRI. In addition, phages in group C (isolates No. 2 and 7) showed the same DNA restriction pattern with EcoRI and their genomic size was found to be 23.800 kbp.

Therefore, on the basis of the above mentioned information, the phages of each group represent one phage type. *i.e.* the ninephage isolates of *Azospirillum*were found to be belonging to three phage types. The three phage types of *Azospirillum*were designated as∅Az1, ∅Az2 and ∅Az3 for phages of group A, B and C, respectively.

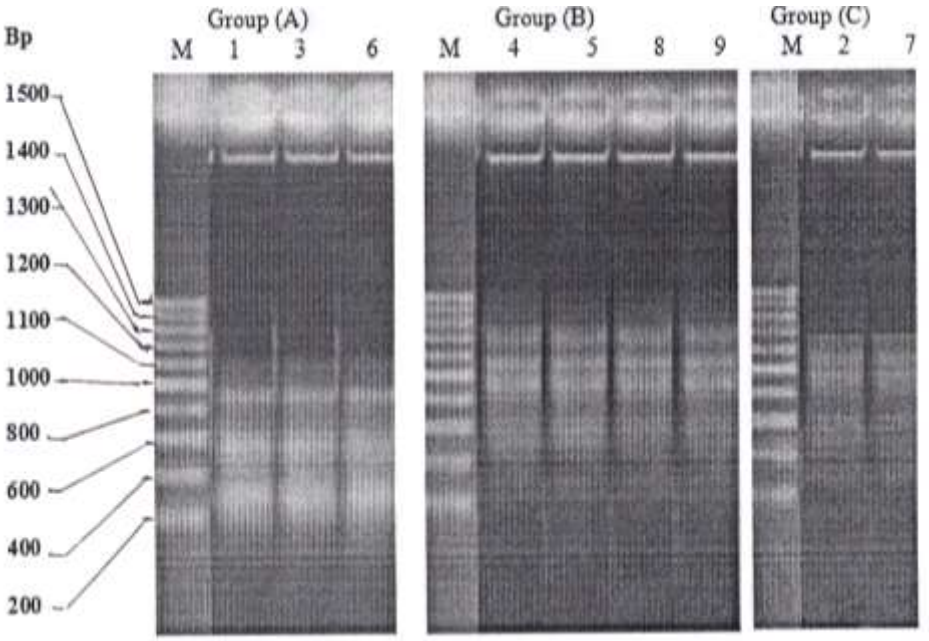

Figure (3):Electrophoresis of EcoRI DNA restriction fragments of bacteriophages specific to *Azospirillum* classified in groups A, B and C.M = Marker DNA

Table (5): The estimated genome size (kbp) of *Azospirillum* phage isolates.

Phage type	∅Az1			∅Az2				∅Az3	
Group	A			B				C	
Phage No.	1	3	6	4	5	8	9	2	7
Genome size	22.57	22.57	22.57	24.51	24.51	24.51	24.51	23.80	23.80

f- Size and morphology of phage particles:

Bacteriophage particles of each phage type specific to*Azospirillum* sp. were negatively stained and examined by electron microscope. All phage types were found to be of head and tail type (Figure 4). On the basis of the phage particle morphology and dimensions (Table 6), the three phage types of *Azospirillum* sp. were found to be of non-contractile tails. Phage type ∅Az1 had isometric head of 76 ± 3 nm in diameter and the shortest tail (38 ± 2 nm) among the three phage types. The other two phage types (∅Az 2 and ∅Az 3) were of long tails and isometric heads.

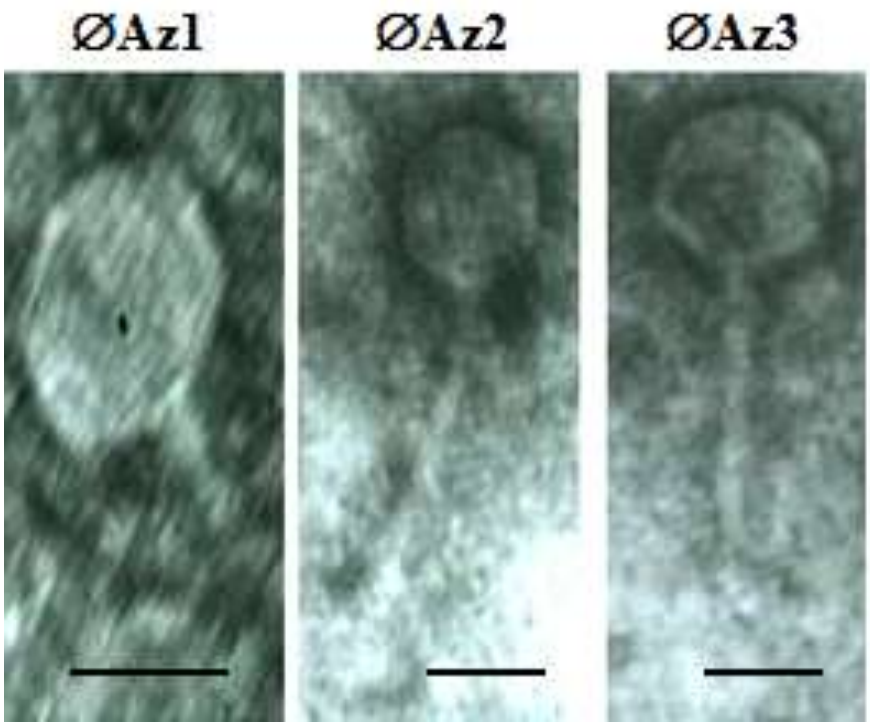

Figure (4): Electron micrographs of negatively stained particles of phage types specific to *Azospirillum* sp. Magnification bar = 50 nm

Table (6): Dimensions***** of bacteriophage particles specific to *Azospirillum* sp.

Phage type	Head diameter ±SD (nm)	Tail	
		Length±SD (nm)	Width±SD (nm)
∅Az1	76 ± 3	38 ± 2	7 ± 3
∅Az2	60 ± 2	150 ± 3	10 ± 2
∅Az3	75 ± 4	147 ± 2	*8 ± 3*

SD = Standard deviation

***** Dimensions represent the average of four particles.

g- One step growth curve:

One step growth curve experiment was carried out for each phage type. As shown in figure (5) the latent periods (defined as the time interval between the absorption and the beginning of the first burst) for ∅Az1, ∅Az2 and ∅Az3 were found to be 10 min, 20 min and 15 min, respectively. Moreover, the burst sizes (the ratio of the final count of liberated phage particles to the initial count of infected bacterial cells during the latent period) for ∅Az1, ∅Az2 and ∅Az3 were

estimated to be 75 pfu/cell, 93 pfu/cell and 82 pfu/cell, respectively.

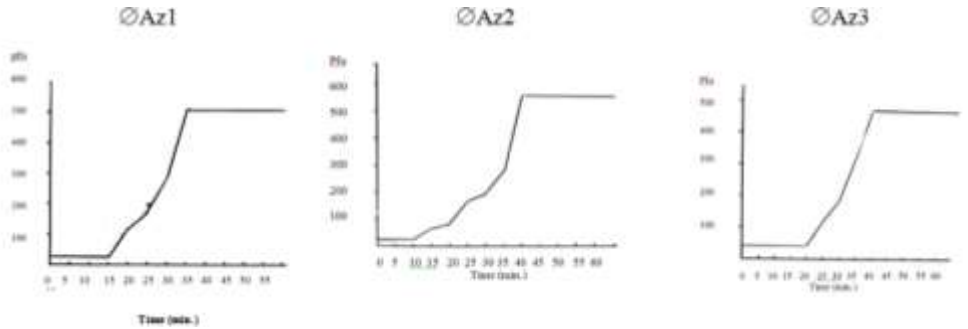

Figure (5): One step growth curveof *Azospirillum* bacteriophages (∅Az1, ∅Az2 and ∅Az3)

DISCUSSION

Bacteriophages specific to *Azospirillum* were successfully isolated from rhizosphere soil sample ofwheat plants and were found to be common in the soils from where the sample had been taken. Similar results were obtained by Fathy (2004) andElsharouny (2007), who isolated phages of *Azospirillum*from rhizosphere soils of different plants.

Since it is assumed that each plaque has originated from the progeny of a single phage particle (Kiraly, *et al.,* 1970). The single plaque isolation technique was used to purify phages. Nine single plaques of phages specific to *Azospirillum sp.* having different morphologies were picked and kept as single pure phage isolates. The isolated phages of formed circular single plaques of 1 to 4 mm in diameter and their appearance varied from hazy to clear.

One hundred ml of high titer phage suspension was prepared for each phage isolate of *Azospirillum.* The titers of the prepared phage suspension ranged from 10^{10} to $6.3x10^{12}$ pfu/ml. Such high concentrations of phages were not surprising, since a single plaque of 2mm in diameter may contain between 10^{7} and 10^{9} recoverable phage particles (Gunsalus and Stanier, 1960 and Adams, 1966).

Since, the single plaques of the nine phage isolates of *Azospirillum* were morphologically different, it was expected that each single phage isolate represents one phage type. *i.e.* the isolated phages may be nine different phage types. In order to assess this expectation, the different characteristics of the isolated phages were studied.

The infectivity of the nine phage isolates of *Azospirillum* was studied at various pH levels (pH 4 – 12). All isolated bacteriophages were found to be tolerant to alkaline and acidic reactions. Similar results were

optained by Elsharouny (2007). The optimum pH for any of the isolated phages was found to be pH 8.

Since, the optimum pH for the nine phage isolates was found to be the same (pH 8), these results may indicate that: 1- The nine phage isolates are belonging to one phage type. 2- The nine phage isolates may be different phage types and all have the same optimum pH. Such explanation seems to be the most acceptable since, Elsharouny (2007) isolated different phage types specific to *Azotobacter*and *Azospirillum*and all had the same optimum pH (pH 8).

Therefore, further characterizations for the phage isolates were needed to be carried out, to accept or dismiss any of the above mentioned hypotheses.

The thermal inactivation point of each phage isolate was estimated. According to the thermal inactivation point results, the nine phage isolates of *Azospirillum* were classified into three groups. Group A comprised three phage isolates (No. 1, 3 and 6) which they were exhibited the same thermal inactivation point (90ºC). Group B contained four phage isolates (No. 4, 5, 8 and 9) of the same thermal inactivation point (55ºC). Group C contained phage isolates No. 2 and 7 of the same thermal inactivation point (60ºC). The thermal inactivation point was used by many investigators as a characteristic of phage isolates **(**Aharchi, 1992; Hammad, 1993; Hammad*et al*., 1995; Othman, 1997; Hammadand Ali, 1999).

On the basis of the above mentioned results, different hypothesis could be possible: first: The nine phage isolates are likely to be three phage types. Hammad (1993) and Hammad and Ali (1999) stated that the different phage types of *B. japonicum*ex habited had different thermal inactivation point. Moreover, Abo-Sinna (2004) reported that the thermal inactivation points for four phages of *Bacillus subtilis* ranged between 50 - 80°C. Elsharouny (2007) reported that phages of either *Azotobacter*or *Azospirillum*which are belonging to one phage type were found to have the same thermal inactivation point. Secand: Since the phage isolates were different in their plaque morphology, each phage group may comprise phage isolate belonging to more than one phage type but all exhibited the same thermal inactivation point and the same optimum pH.

To accept one of the above hypothesis further studies were carried out. Sensitivity of the isolated phages of *Azospirillum* to U.V. radiation (at wave length of 260) was studied. According to the sensitivity to U.V. radiation, the nine phage isolates were divided to three groups. Interestingly, the three phage groups of *Azospirillum*, which were divided on the basis of the thermal inactivation point were found to be the same as those classified on the basis of the sensitivity to U.V. radiation.

Such results may indicate that the phages of each group may represent a single phage type. Similarly,

Elsharouny (2007) found that phage isolates of either *Azotobacter*or *Azospirillum*which both belonging to one phage type were found to have the same sensitivity to U.V. radiation.

To confirm this explanation, additional characterizations for the isolated phages were carried out, *e.g*. longevity *in vitro* as well as DNA restriction pattern using EcoRI.

According to the results of longevity *in vitro* study, the nine phage isolates were classified into three groups. Interestingly, these groups of phages, which were divided on the basis of the thermal inactivation point and sensitivity to U.V. radiation were found to be the same as those classified on the basis of the longevity *in vitro*.

The DNA restriction patterns of the isolated phages were studied and the genome size of each phage isolate was estimated. The results indicated that phages of each group which were classified on the basis of the above features exhibited identical restriction patterns and the same genome size.

Therefore, on the basis of the above mentioned information, it can be concluded that the phages of each group represent one phage type. *i.e.* phages of *Azospirillum*were found to be belonging to three phage types. The three phage types of *Azospirillum*were designated ∅Az1(phages of group A), ∅Az2 (phages of group B) and ∅Az3 (phages of group C).

Each bacteriophage type specific to *Azospirillum*(∅Az1, ∅Az2 or ∅Az3) was negatively stained and examined by electron microscope. The three phage types were found to be of head and tail types. The three types were of non contractile tails and isometric heads. ∅Az1 was found to be a member of family*Podoviridae*. Whereas, ∅Az2 and ∅Az3 were found to be belonging to family *Siphoviridae.* Similarly, Elsharouny (2007) isolated phages specific to *Azospirillum* of head and tail types.

One step growth curve experiment was carried out for each phage type. The latent period of each phage type was estimated and was found to be 10 min, 20 min and 15 min and burst size was calculated to be 75 pfu/cell, 93 pfu/cell and 83 pfu/cell for ∅Az1, ∅Az2 and ∅Az3, respectively. Similarly, Ahmady*etal*. (2016) reported that the latent period of phages Ec02 and Ec02 of *Entrerobactercloacae* was estimated to be 15 min.

CONCLUSION

Generally, in this study different characteristics were studied all together to differentiate and classify the isolated phages of *Azospirillum.* No single technique for characterizing phages is in itself sufficient for complete identification or classification, but these features (the optimum pH, thermal inactivation point, sensitivity to U.V. radiation, longevity in vitro and DNA restriction pattern) must be studied all together to differentiate between the isolated phages.

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الملخص العربي

عزل وتوصيف الفيروسات البكتيريه المتخصصه على بكتيريا الازوسبيريلام

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تم عزل تسعة فاجات متخصصة على بكتيريا الازوسبيريلم بنجاح من ريزوسفير نباتات القمح،المنزرعة في المزرعة التجريبية بكلية الزراعة، جامعة المنيا، المنيا، مصر. تم دراسة الخصائص المختلفة لهذه الفاجات المعزولة (درجة الاس الهيدروجينى المثلى للعدوى، ودرجة التثبيط الحرارى، والحساسية لأشعةU.V ، فترة البقاء فى درجة حرارة الغرفه، وناتج التفريد الكهربى للجينوم المهضوم بانزيم EcoR1) وذلك للتمييز والتفريق بينالفاجات المعزولة. على أساس الاختلافات في هذه الصفات المدروسة لهذه الفاجات، تم تقسيم الفاجات التسعة إلى ثلاث مجموعات (A، B، و C). قد أظهرت فاجات كل مجموعة نفس الصفات. لذلك، اعتبرت الفاجاتلكل مجموعة نوع واحد من الفاجات. أي العزلات التسعة ينتمون إلى ثلاثة أنواع. وقد تم تسمية أنواع الفاجاتالثلاثة الىAz1∅، Az2∅ وAz3∅ لفاجات المجموعاتA و B و C، على التوالي.

تم فحص كل أنواع الفاجات بواسطة الميكروسكوب الالكتروني. وقد ظهر أن جميع أنواع الفاجاتمن النوع الذى يحتوى على رأس وذيل. تم العثور على نوع Az1∅ينتمي إلى عائلة فيروسات *Podoviridae*. في حين، تم العثور على Az2∅ وAz3∅ تنتمي إلى عائلة فيروسات *Siphoviridae*. وقد أجريت تجربة منحنى النمو ذو الخطوة الواحدة لكل نوع من الفاجات. وقدرت فترة الكمونلكل نوع من الفاجات ووجد أنها 15 دقيقه ، 10دقاق و 20 دقيقة على التوالى ، كما تم حسابحجم الانفجاروتبين انه 75 فاج / خلية، 93 فاج / خلية و 82 فاج / خلية لانواع الفاجات Az1∅، Az2∅ وAz3 ∅ على التوالي.