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BACTERIOPHAGES SPECIFIC TO *BACILLUS SUBTILIS* I- ISOLATION AND CHARACTERIZATION

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ABSTRACT

Ten single lytic phage isolates specific to *Bacillus subtilis* were successfully isolated from rhizosphere soil of wheat plants, growing in the Experimental Farm of Faculty of Agriculture, Minia University, Minia, Egypt. The different characteristics of the ten phage isolates were studied, *i.e.* host specificity, the optimum pH for viral infection, thermal inactivation point, sensitivity to UV radiation, particle size, and morphology. The isolated phages exhibited tolerance to alkaline and acidic reactions. All phage isolates were found to be of head and tail types. On the basis of the differences in the studied characteristics of these phages, the ten phage isolates were divided into five groups (A, B, C, D and E). Phages of each group exhibited the same features (*i.e.* thermal inactivation point, sensitivity to UV radiation, as well as phage particle size and morphology). Accordingly, the ten phage isolates are belonging to five phage types. These five phage types were designated ∅Bs1, ∅Bs2, ∅Bs3, ∅Bs4 and ∅Bs5. Specific antiserum was successfully obtained for each phage type via immunization of white New Zealand rabbits. Slide agglutination test indicated that the isolated phage types were found to have the immunogenicity and antigencity properties. All phage types showed heavy agglutination with their specific antisera and no agglutination with normal serum. The double immunodiffusion test indicated that the five phage types were found to be serologically related to each other.

Key words: *Bacillus subtilis*, bacteriophage, UV radiation, rhizosphere, antiserum.

INTRODUCTION

Soil fertility improvement is one of the most common tactics to increase agricultural production. Chemical fertilizer is widely used to supply essential nutrients for plant to increase yield. In fact, yield of most crop plants are increased linearly with the amount of fertilizer that they absorb. Micro-organism employed to enhance the availability of nutrients, called biofertilizer. Biofertilizers are commonly used as alternatives to chemical fertilizers for increasing soil fertility and plant growth in sustainable agriculture, thus received vast attention in recent times. They can solubilize insoluble phosphate and produces plant growth promoting substances such as IAA, amino acids, vitamins etc. (Principe, *et al*., 2007; Kumar, *et al*., 2011). *Bacillus* species are a major component of the microbial flora, which live in close association with various types of agricultural crops. Predominance of *Bacillus* sp. is due to its ability to efficiently use the nutrients provided by the plant through exudates (Joshi and Bhatt, 2011). *Bacillus subtilis* is one such commercialized organism and it acts against a wide variety of pathogenic fungi. These bacteria competitively colonize the roots of plant and can act as biofertilizers or antagonists (biopesticides) or simultaneously both (Hurek and Reinhold-Hurek 2003). Use of these microbes as fertilizers in the field has been reported to increase crop growth and yield.

For such economically important microorganisms (*Bacillus subtilis*), knowledge of factors influencing the survival, establishment and activities of these desired bacteria in the soil is of a particular interest. Therefore, presence of bacteriophages is likely to be one of the most important factors' influencing the maintenance and activities of these bacteria. Presence of bacteriophages specific to *Bacillus megaterium* had a depressive effect on the efficiency of these bacteria in disscving phosphate (Zayed, 1998 and Hammad, 1999).It is well known that bacteriophages are of widespread occurrence and are usually found in soils which contain the appropriate bacterial host. Therefore, presence of phages in the soils may explain the observation concerning biofertilization failure on several plants grown at different localities, even when different local or foreign inocula of high efficiency were used.

Efforts were made to characterize and identify the bacteriophages of *B. subtilis* on the basis of plaque morphology and particle size and morphology (Fathy, 2008 and Farahat, 2016). However, the number of the studied phages was too few and the details given are too limited

This work aimed to study the occurrence of bacteriophages specific to *B. subtilis* in Minia soil. Moreover, the different characteristics of the isolated phages (*i.e.* optimum pH for

infection, thermal inactivation point, sensitivity to UV radiation, host range, particle size, and morphology) were also studied to characterize and differentiate the phage isolates.

MATERIALS AND METHODS

1- **Bacterial isolate:** Identified *B. subtilis* isolate which used in this study, was kindly provided by Cairo MIRCEN (Microbial Resource Center), Faculty of Agriculture, Aim Shams University, Cairo, Egypt.

2- **Source of Bacteriophages:** A soil sample was randomly collected from the rhizosphere of wheat plants, growing in the Experimental Farm of the Faculty of Agriculture, Minia University, Minia, Egypt, to be used as a source of bacteriophages.

3- Isolation of Bacteriophages: The liquid enrichment technique of Adams (1966) was used to isolate phages specific to *B. subtilis* from the collected rhizosphere soil sample as described by Barnet (1972).

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

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

c- 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) and Farahat (2016).

d- Titer Estimation: Titer was estimated using the method described by Kiraly *et al.* (1970). From the phage suspension, a series of tenfold dilution was prepared in sterile eppendorf vials. The dilutions were prepared by measuring 90 µl of SM medium (Maniatis *et al.*, 1982) into each vial. Ten µl of phage suspension were added to the first vial and mixed, then 10 µl from the first vial were transferred into the second one and so on, until the last vial. After dilution, 200 µl of indicator bacterial suspension were placed in each vial. The contents of each tube were shaken and transferred to a sterile test tube containing 3 ml of melted nutrient agar semi-solid medium (0.7% ager), which had been prepared before and kept at 50-55 °C. Each tube was shaken separately, and the contents were poured onto previously prepared solid medium plates, then they were incubated at 30-33°C for 24 h. The formed plaques were counted and the titer was calculated and expressed as plaque forming unit (pfu)/ml.

4- Characterization of bacteriophages:

a- The optimum pH level: Nine eppendorf tubes each containing 1 ml SM media with various pHs (*i.e.* 4 up to 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 10µl from each tube were spotted over double agar layer plates (three replicates), containing *B.subtilis*, followed by incubation at 30-33ºC for 24-48 h. Diameters of the lysed spots were measured. The average values f the replicates was calculated.

b- Host range Assay: The host range of each phage isolate was determined using the spot test technique. Each of the phage isolates of *B. subtilis* was tested against *B.subtilis*, *B. cereus, B. megaterium* and *B. polymyxa*

c- Thermal inactivation point: Ten eppendorf tubes each containing 1 ml 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 *B. subtilis* as indicator bacteria. Plates were inspected for lysed spots after 24-48 hrs incubation at 30-33°C.

d- Sensitivity to ultraviolet radiation: Five ml of high titer phage suspension of each single phage isolate were put in dishes placed at distance of 20 cm from UV lamp of 254 nm wavelength. Ten µl of each irradiated phage suspension were spotted over double agar layer plates that containing the *B. subtilis* as indicator bacteria, after 10, 20, 30, 40, up to 90 min. exposure to UV irradiation. Plates were inspected for lysed spots after incubation for 24-48 hrs at 30-33°C.

e- 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.

5- Serological studies

a- Production of antisera specific to *B. subtilis* phages: Three white New Zealand rabbits each about 2.5 kg were used to produce antiserum for each phage type. Prior to rabbits immunization, preimmune serum (normal serum) was collected as a control. The immunization schedule for production of the specific antisera for phage isolates was used as described by Marei, *et al.* (2014).

b- Serological properties of *B. subtilis* phages: The serological properties were studied using slide agglutination test as described by El-Safty (2016) and double immunodiffusion test in agar matrix as described by Ouchterlony (1968).

RESULTS

Occurrence of bacteriophages specific to *B. subtilis* in the collected Soil sample:

Bacteriophages specific to *Bacillus subtilis* were successfully isolated from the soil sample collected from rhizosphere soil wheat plants, growing in the Experimental Farm of the Faculty of Agriculture, Minia University, Minia, Egypt,. 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 *Bacillus subtilis* were found to be common in the collected soil sample.

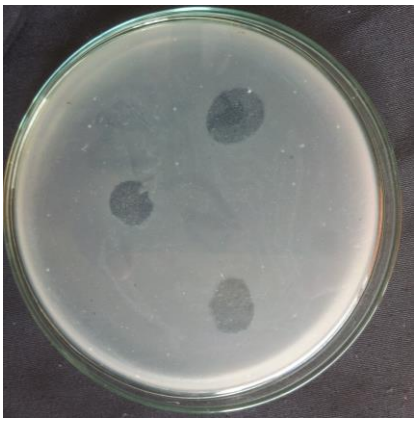

Figure (1): A bacterial lawn of *B. subtilis*, spotted with drops of the prepared phage lysate and incubated for 24-30 hrs at 30 °C. The lysed spots are clearly seen.

Purification of phages

The single plaque isolation technique was used to obtain pure phage isolates of *B. subtilis*. As shown in Figure (2) the phages specific to *B. subtilis* formed single plaques of different morphologies. Ten single plaques morphologically different were selected and kept as pure phage isolates. The isolated phages formed circular single plaques of 1 to 3 mm in diameter and clear in appearance.

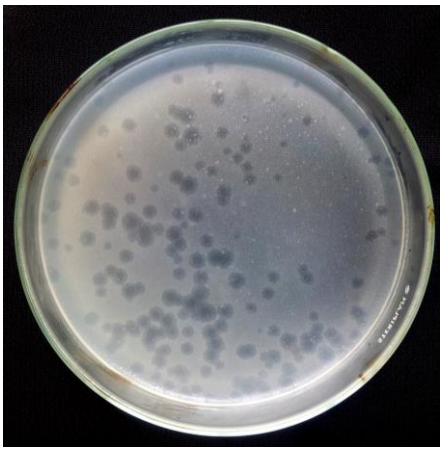

Figure (2): A plate containing single plaques of bacteriophages specific to *B. subtilis*. The differences in morphology of the single plaques are clearly seen.

The high titer phage suspensions: One hundred ml of high titer phage suspension were prepared for each phage isolate of *B. subtilis*. The titers of the prepared suspensions of the ten phage isolates specific to *B. subtilis* were ranged from $2.7x10^{10}$ to $4.5x10^{12}$ pfu/ml.

Characteristics of the isolated phages: The different characteristics of the ten phage isolates of *B. subtilis* were studied to find out if these phage isolates are different types or similar.

Host range of the isolated Phages:

Each of the ten phage isolates of

B. subtilis was tested against four different bacillus species. As shown in Table (1), all phage isolates were infectious to *B. subtilis* (the main host). Whereas, none of the ten phages was infectious to the other tested *Bacillus* species.

Table (1): The host range of the phage isolates specific to *B. subtilis*.

	Bacillus spp.			
	Bacillus subtilis	*Bacillus cereus*	*Bacillus megaterium*	*Bacillus polymyxa*
1	+	-	-	-
2	+	-	-	-
3	+	-	-	-
4	+	-	-	-
5	+	-	-	-
6	+	-	-	-
7	+	-	-	-
8	+	-	-	-
9	+	-	-	-
10	+	-	-	-

+ = Lysis - = No lysis

The optimum pH for phage infection: The infectivity of the ten phage isolates of *B. subtilis* was studied at various pH levels (pH 4-12). As shown in Table (2), all phage isolates formed lysed spots at any pH level (pH 4-12). Whereas, all phage isolates formed widest spots at pH 9.

Table (2): Stability of bacteriophages specific to *B. subtilis* to different pH levels

Phage No.	PH levels								
	4	5	6	7	8	9	10	11	12
	Diameter of the lysed spots (mm.)								
1	10.4	11.2	11.4	13.3	14.4	16.8	16.2	16.0	10.2
2	9.1	10.4	13.3	14.4	18.4	19.3	15.5	16.2	10.2
3	8.8	8.9	11.3	12.4	14.7	18.7	16.2	15.5	11.1
4	10.1	11.4	14.3	15.3	16.4	18.1	16.2	11.3	11.0
5	8.2	9.8	11.3	15.3	14.1	16.6	12.4	8.8	8.3
6	10.9	11.5	12.4	13.4	14.9	**19.1**	16.8	15.4	10.0
7	12.3	13.5	14.2	15.3	15.1	**16.5**	16.0	11.1	10.3
8	14.7	14.5	13.8	13.4	16.5	**17.1**	16.3	13.6	13.0
9	6.9	7.8	9.9	16.8	17.0	**17.4**	14.5	13.4	10.0
10	6.2	6.6	7.1	7.5	7.7	**8.8**	8.0	7.8	5.6

The underlined numbers indicate the optimum pH

Thermal inactivation point of the phage isolates:

Data presented in **Table** (3) indicate that the ten phage isolates of *B. subtilis* were grouped into five groups (A, B, C, D and E). Group (A) comprised two isolates (isolates No. 1 and 3) of the same

thermal inactivation point (95°C). Whereas, group B contained two isolates (No. 8 and 9). The thermal inactivation point of these two phage isolates was found to be 75°C. The thermal inactivation point of of Group (C), which comprised three phage isolates (No. 2, 4 and 5) was found to be 70°C. Group (D) contained one phage isolate (No. 7) of the thermal inactivation point (65°C). Group E contained two phage isolates (No. 6 and 10) of the same Thermal inactivation point (85 °C).

Table (3): Thermal inactivation points of bacteriophages specific to *B. subtilis*.

Phage group	Phage No.	Temperature (°C)									
		50	55	60	65	70	75	80	85	90	95
A	1	+	+	+	+	+	+	+	+	+	-
	3	+	+	+	+	+	+	+	+	+	-
B	8	+	+	+	+	+	-	-	-	-	-
	9	+	+	+	+	+	-	-	-	-	-
C	2	+	+	+	+	-	-	-	-	-	-
	4	+	+	+	+	-	-	-	-	-	-
	5	+	+	+	+	-	-	-	-	-	-
D	7	+	+	+	-	-	-	-	-	-	-
E	6	+	+	+	+	+	+	+	-	-	-
	10	+	+	+	+	+	+	+	-	-	-

+ = Lysis - = No lysis

Sensitivity to Ultraviolet Irradiation:

As shown in **Table** (4), the UV radiation at wavelength of 254 nm inactivated the isolated phages at different exposure times. Accordingly, the isolated phages of *B. subtilis* were divided into five groups (A, B, C, D and E). Group (A) comprised two isolates (isolates No. 1 and 3), which were inactivated after 80 min exposure to UV radiation. Whereas, group (B) which comprised two phage isolates (isolates No. 8 and 9) was inactivated after exposure to UV for 70 min. Phage isolates No. 2, 4 and 4 which formed group (C) was inactivated after exposure to UV for 90 min. In addition, phage isolate No. 7 which formed group (D) were inactivated after 60 min. exposure to UV radiation. Moreover, group E comprised two phage isolates (No. 6 and 10). These two phages kept their infectivity even after exposure to UV for 90 min.

Table (4): Effect of UV radiation (wave length 254 nm) on bacteriophages specific to *B.* subtilis.

Groups of Bacteriophages	Phage No.	Exposure time (min.)								
		10	20	30	40	50	60	70	80	90
A	1	+	+	+	+	+	+	+	-	-
	3	+	+	+	+	+	+	+	-	-
B	8	+	+	+	+	+	+	-	-	-
	9	+	+	+	+	+	+	-	-	-
C	2	+	+	+	+	+	+	+	+	-
	4	+	+	+	+	+	+	+	+	-
	5	+	+	+	+	+	+	+	+	-
D	7	+	+	+	+	+	-	-	-	-
E	6	+	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	+	+

+ = Lysis - = No lysis

Size and morphology of phage particles: Bacteriophage isolates specific to *B. subtilis* were negatively stained and examined by electron microscope. All phage isolates were found to be of head and tail type (**Figure** 3). As shown in **Table** (5), on the basis of the phage particle dimensions the phage isolates No. 1 and 3 were found to be similar in their head diameters as well as in length and width of their tails. Therefore, these two phage isolates formed one group (group A). Moreover, phages No. 8 and 9 were also classified in another group (group B), since they exhibited similar dimensions. Moreover, group C contained phage isolates (phage No. 2, 4 and 5). In addition, phage No. 7 was also classified in group D. Moreover, phages No. 6 and 10 were found to be similar in their dimensions and formed group E. phages of each group exhibited similar particle dimension. On the basis of these results, the phage isolates of each group represent one phage type. *i.e.* the ten phage isolates are belonging to five types. These five phage types were designated ∅ Bs1, ∅ Bs2, ∅ Bs3, ∅ Bs4 and ∅ Bs5.

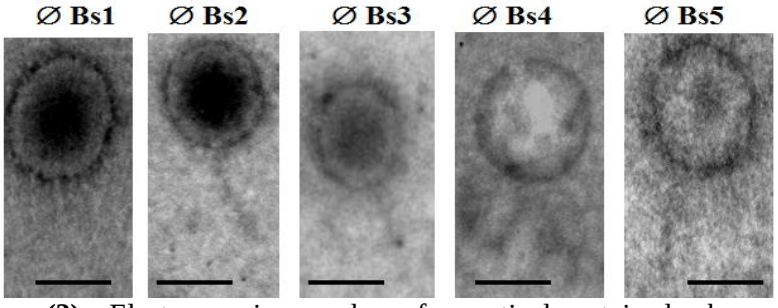

Figure (3): Electron micrographs of negatively stained phage particles specific to *B. subtilis*. Magnification bar = 50 nm

Table (5): Dimensions***** of bacteriophage particles specific to *B. subtilis*

Phage group	Phage No.	Head diameter ± SD (nm)	Tail	
			Length ± SD (nm)	Width ± SD (nm)
Phage isolates of *Bacillus subtilus*				
A	1	84 ± 2	38 ± 3	8 ± 2
	3	82 ± 3	36 ± 4	7 ± 2
B	8	77 ± 3	115 ± 2	11± 3
	9	75 ± 2	113 ± 3	10 ± 2
C	2	67 ± 3	46 ± 2	6 ± 3
	4	63 ± 4	44± 3	5 ± 2
	5	65±3	42±2	4±3
D	7	80 ± 2	65 ± 3	15 ± 2
E	6	86 ± 4	84± 3	10 ± 4
	10	83±3	82±2	9±3

SD = Standard deviation * Dimensions represent the average of four particles**.**

As shown in Figure (4) the isolated phage types were found to have the immunogenicity and antigencity properties. All phage types showed heavy agglutination with their specific antisera and no agglutination with normal serum was detected.

Serological properties to *B. subtilis* phage isolates

Specific antiserum was obtained for each phage type when white New Zealand rabbits were immunized with the isolated phages using subcutaneous and intramuscular injections.

Slide agglutination test

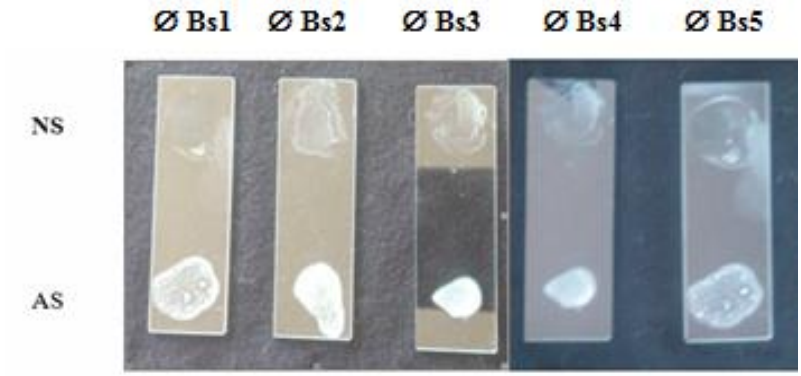

Figure (4): Slide agglutination test for the five isolated phage types.
NS = normal serum As = Antiserum

Double immunodiffusion test (Ouchterlony test):
The double immunodiffusion test was carried out to determine the serological relationships between the five phage types under study. As shown in Figure ( ) the antiserum prepared for phage ∅Bs1 reacted with ∅Bs1 and ∅Bs4 as well as ∅Bs5. This result may indicates that ∅Bs1 is serologically related to ∅Bs4 and ∅Bs5. Moreover, each antiserum produced for ∅Bs2, ∅Bs3, ∅Bs4 or ∅Bs5 reacted with its own phage indicated by formation of clear precipitation line between the anigen and antiserum wells. As shown in Figure (5) ∅Bs2 is serologically related to ∅Bs1, ∅Bs 4 and ∅Bs5. Generally, the five phage types were found to be serologically related to each other.

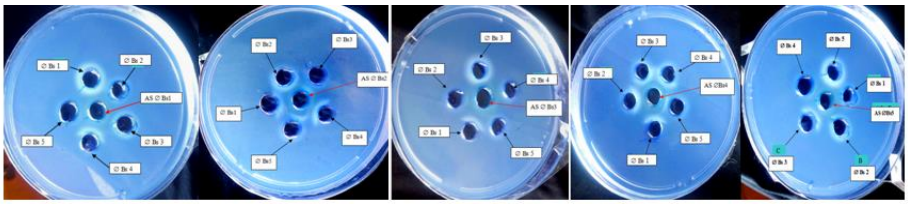

Figure (5): Determination of serological relationships between the five phage types via double immunodiffusion test. The central wells contain antisera and peripheral wells contain suspensions of ∅Bs1, ∅Bs2, ∅Bs3, ∅Bs4 and ∅Bs5 (clock wise).

DISCUSSION

Bacteriophages of *B. subtilis* were successfully isolated from the rhizosphere of wheat plants, growing in the Experimental Farm of the Faculty of Agriculture, Minia University, Minia, Egypt, and were found to be common in the soil from where the soil sample had been taken. Similar results were obtained by Zayed (1998); Fathy (2004) and Elmaghraby *et al*. (2015), who isolated phages of *B. megaterium* 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 and Elmaghraby *et al*., 2015). The single plaque isolation technique was used to purify phages. Ten single plaques of phages specific to *B. subtilis* having different morphologies were picked and kept as single pure phage isolates. The isolated phages of *B. subtilis* formed circular single plaques of 1 to 3 mm in diameter and clear in appearance.

It is commonly believed that the shape, size and outline of the plaques are characteristic of the phage strain **(**Marei and Elbaz, 2013 and Elmaghraby *et al*., 2015). Barnet (1972) reported that the isolates of *Rhizobium trifolii*

phages of the same morphological type had similar plaque characteristics. Moreover, Elsharouny (2007) isolated different phage types specific to *Azotobacter* spp. and *Azospirillum* spp. Each phage type formed single plaques of similar morphology.

One hundred ml of high titer phage suspension was prepared for each phage isolate of *B. subtilis* using plate method as described by Maniatis *et al*. (1982). The titers of the prepared phage suspension were ranged from $2.7x10^{10}$ to $4.5x10^{12}$ pfu/ml. Such high concentrations of phages are not surprising, since a single plaque of 2 mm in diameter may contain between 10^7 and 10^9 recoverable phage particles Elsharouny, 2007 (Fathy, 2008; and Elmaghraby *et al*., 2015).

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

Each of the ten phage isolates of *B. subtilis* was tested against four different bacillus species. All phage isolates were infectious to *B. subtilis* (the main host). Whereas, none of the ten phages was infectious to the other tested bacillus species. Barnet (1972) stated that the ability of phage particle to lyse a bacterial strain is dependent upon the presence of certain micro-molecules on the surface of the cells, *viz* surface receptors for bacteriophage adsorption.

Hammad and Ali (1999) studied the host range of 60 phage isolates specific for *B. japonicum*. Based on host ranges the 60 phage isolates were classified into 4 phage groups. Each group comprised the phage isolates of the same host range. Kankila and Lindstrom (1994) studied the host range of 11 bacteriophages specific to *R. leguminosarum* bv *trifolii* using 32 *Rhizobium* strains. They concluded that three morphological types were found among the phage isolates. Type 1 phages were able to lyse all bacterial strains tested whereas type 2 and 3 exhibited narrower host range. On the basis of the host range results different hypotheses could be possible: **1**- The ten phage isolates may be belonging to one phage type since, the ten phage isolates exhibited the same host range. 2- The ten phage isolates belong to different types and all have the same host range. Therefore, to accept one of these hypotheses further characteristics for the ten phage isolates were studed.

The infectivity of the ten phage isolates of *B. subtilis* 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 obtained by Elsharouny (2007). The optimum pH for infection was studied for

each phage isolate. The optimum pH for the isolated phages was found to be the same (pH 9) On the basis of the optimum pH, the ten phage isolates may be belonging to one phage type or may be belonging to different phage types and all have the same optimum pH (pH 9). To accept one of these postulates, further studies were carried out.

The thermal inactivation point of each phage isolate was estimated. According to the thermal inactivation point results, the ten phage isolates were divided to five groups. Phage isolates of each group were found to have the same thermal inactivation point. Similarly 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. Abo-Sinna (2004) reported that the thermal inactivation points of four phages of *B. subtilis* ranged between 50-80°C.

Moreover, sensitivity of the ten isolated phages of *B. subtilis* to UV radiation (at wavelength of 254) was studied. According to the sensitivity to U.V. radiation, the ten phage isolates were divided into five groups. Interestingly, the five phage groups of *B. subtilis*, 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 UV 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 belonging to one phage type, were found to have the same sensitivity to UV radiation.

To confirm this explanation, additional characterizations for the isolated phages were carried out, *e.g*. size and morphology of each phage isolate.

Bacteriophage isolates specific to *B. subtilis* were negatively stained and examined by electron microscope. All phage isolates were found to be of head and tail type. Similarly, Elmaghraby *et al*. (2015) isolated phages specific to *B. megaterium* of head and tail type. Interestingly, phages of each group (divided on the basis of the thermal inactivation point, and sensitivity to UV radiation were found to be morphologically similar. The differences in head and tail dimensions of the phages of each group are within the standard deviation and are not statistically significant. Francki (1973) reported that since there are a number of unknown factors able to affect particle size during various preparative procedures, it is difficult to make valid comparisons between published morphometric data. Accordingly, morphological differences do not necessarily imply differences in phages of each group but because these phages of each group showed the same thermal inactivation point and the same sensitivity to UV radiation, the phages of each group must be belong to a single phage type. *i.e.* five phage types were isolated

specific to *B. subtilis* and designated ∅Bs1, ∅Bs2, ∅Bs3, ∅Bs4 and ∅Bs5.

Specific antiserum was successfully obtained for each phage type via immunization of white New Zealand rabbits using subcutaneous and intramuscular injection as described by Marei, *et al.* (2014) and El-Safty (2016). Slide agglutination test indicated that the isolated phage types were found to have the immunogenicity and antigencity properties. All phage types showed heavy agglutination with their specific antisera and no agglutination with normal serum was detected. Similar results were obtained by Marei *et al.* (2017). The double immunodiffusion test indicated that the five phage types were found to be serologically related to each other.

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الفيروسات البكتيرية المتخصصة على *Bacillus subtilis*

1- عزل و توصيف

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في هذه الدراسة تم عزل عشرة عزلات من الفيروسات البكتيرية المتخصصة على بكتيريا *Bacillus subtilis* من تربة منطقة جذور نبات القمح النامي في المزرعة البحثية لكلية الزراعة جامعة المنيا.

تم دراسة الخصائص المختلفة للفاجات المعزولة مثل المدى العوائلى ودرجة تركيز أيون الهيدروجين الأمثل للإصابة ودرجة التثبيط الحرارى والحساسية للأشعة فوق البنفسجية ومرفولوجيا وأبعاد الجزيئات الفيروسية. أظهرت الفيروسات المعزولة تحمل لظروف الحموضة والقلوية. كما ان الفاجات المعزولة تبين أنها من النوع ذات الراس والذيل.

اعتمادا على الاختلافات في الخصائص التي تم دراستها فقد قسمت العشرة عزلات من الفيروسات إلى خمسة مجموعات (A, B, C, D, E). تضمنت كل مجموعة الفيروسات ذات الخصائص المتشابهة.

وبناء على ذلك فقد خلصت الدراسة إلى أن العشرة عزلات من الفيروسات تنتمى إلى خمسة أنواع مختلفة حيث تم تسميتهم ∅Bs1, ∅Bs2,∅Bs3، ∅Bs4 , ∅Bs 5.

تم تحضير السيرم المضاد لكل نوع من الفاجات الخمسة بحقن أرانب من النوع نيوزيلاندى الأبيض ومن الدراسات السيرولوجية قد تبين أن كل من الفاجات الخمسة لها خصائص انتيجينية كما اتضح وجود علاقة سيرولوجية بين الأنواع الخمسة.