CHARACTERIZATION OF SIX BACTERIOPHAGES ACTIVE AGAINST *RHIZOBIUM LEGUMINOSARUM* BV. *VICEAE*

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ABSTRACT

In this study, four temperate (TP) and two lytic (LP) isolates of rhizobiophages specific for *R. leguminosarum bv. viceae* were isolated, (from 22 rhizospheric soil samples and nodules of growing broad bean plants from different locations in Egypt), purified, and their morphological and biological properties as well as some physical factors affecting them were determined. Electron microscopy studies have shown that the tested temperate phages were identical in morphology and had different dimensions, a prolate icosahedral head capsids, short non-contractile tails, and a distail tail knobs. On the other hand, the lytic phages were differentiated in two morphological types, the lytic phage (LP$_2$) had a spherical head, short non-contractile tail and a visible distail tail knob, while the LP$_{11}$ phage had a icosahedral head, short non-contractile tail and non-visible a distail tail knob. Also, the growth parameters studies revealed that all the tested phages exhibited different adsorption rates, maximum adsorption times, percentages of adsorption, averages of burst size, rise periods, but they had, almost, a identical latent periods (60 min.). Different reactions were recorded for the tested phages, when they were tested for their thermal stability at 35 to 75°C for 20 min., their stability at pH ranging from 2.0 to 14.0, their viability to storage periods (30, 60 and 90 days) at low temperatures (below 0 and 4°C), and their sensitivity to UV-irradiation (at 30 cm for 30 min.). In general, the lytic phages (LP$_2$ and LP$_{11}$) were more stable and had higher titers than those of temperate phages (TP$_{27}$, TP$_{88}$, TP$_{97}$ and TP$_{101}$) in most tested factors.

Key words: Rhizobiophages, Morphological types, Biological properties, Physical factors, Electron microscopy

INTRODUCTION

In Egypt, rhizobiophages were found to be common in the Nile valley soils cultivated with leguminous plants, **Emam et al (1983); Othman (1986); El-Didamony (1995); Salama (1992);** **Radwan (1994); Hammad et al (1995); Ali et al (1998) and Hammad and Ali (1999)** and their results indicated that the phage titer was high in the rhizospheric zone, root-nodules and cultures of rhizobia. *Rhizobium* phages are of particular interest because of the ability of their

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hosts to fix atmospheric nitrogen and they are considered of the most biological factors influencing the functions of rhizobia, the process of N₂-fixation and the growth of host plant, **Evans** *et al* **(1979)** and **Barnet (1980)**. Now, it is well known that these phages are often associated with inoculation failure **(Kyukendall and Hashem, 1998** and **Sharma** *et al* 2002). Apart from their importance as a biotic pressure on *Rhizobium*, rhizobiophages have potential applications in (i) the development of ecologically competitive phage-resistant inoculants; (ii) biological control of indigenous infective strains **(Kyukendall and Hashem, 1998 and Sharma** *et al* 2002); (iii) their utility as stable markers for identification, enumeration and tracking of indicator bacterial strains **(Lindstrom** *et al* **1990)**; and (iv) they have transductional ability and used as DNA vectors in genetic engineering studies **(Werquin** *et al* **1989)**. Most of rhizobiophages studies are lytic **(Dhar** *et al* **1987)** although lysogeny was occasionally observed in some rhizobia and with respect to their morphology; these phages were classified in groups A, B and C according to **Bradley (1967)**. The majority of them represent the morphological group B **(El-Wafai** *et al* **1996)**.

Our interest was focused on morphological and biological characterization of rhizobiophages under Egyptian conditions as prerequisite to their use in fundamental and applied research. Therefore, this study was carried out to isolate some temperate and lytic phages specific for *Rhizobium leguminosarum* bv. *viceae*, from the rhizosphere soil of *Vicia faba* plants (and their nodules) grown in some Egyptian soils, characterization the morphological types of six selected rhizo-

phiophages and study the growth parameters, as well as, some physical factors (heating, pH, UV-irradiation and storage period) affecting their activities and titers.

MATERIAL AND METHODS

Rhizobia isolation

A total of 122 rhizobial isolates of *R. leguminosarum* bv. *viceae* were isolated, from 122 root-soil core samples of broad bean plants growing in nine Governorate in Egypt. These isolates were isolated, purified and tested for their plant infection as described by **Somasegaran and Hoben (1985)**. And, only 22 rhizobial isolates were chosen according to their natural growth and the data of the pre-test of plant infection, to be used in this study. The location, type of soil samples, nominations of the rhizobial isolates, and the chosen isolates are given in Table (1).

Rhizobiophage isolation

Isolation of temperate phages

The temperate phage was induced by exposure the growing rhizobia, 122 isolates, counted in YEMB, **Somasegaran and Hoben (1985)**, from 2.0×10^8 to 8.0×10^{10} CFU/ml, to ultraviolet irradiation, **Werquin** *et al* **(1989)**. A 15 watt general electric germicidal lamp 254 nm (Philips) was used for 20 second at a distance of 30 cm. The treated isolates were examined in the lysogeny screening experiment and they were cross-tested in all possible combination with the induced phages by the standard-pot test technique, **Adams (1959)**. Only, eleven temperate phages were obtained, Table (2). *Rhizobium* isolates were classified as sensitive

Table 1. Locations, total no. of soil samples and isolates and the nomination of the chosen isolates

Locations	Total no. of collected samples	Type of soil samples	Total no. of chosen isolates	Nomination
Sharkia	16	Clay	5	S2, 11, 22, 23, 24
	17	Sandy clay loam	4	S26, 27, 36, 112
	14	Loamy sand	1	S113
	1	Sandy	1	S119
Ismailia	7	Loamy sand	1	I41
Fayoum	5	Clay	-	-
	1	Sandy clay loam	-	-
Menoufia	4	Clay	1	M52
	4	Sandy clay loam	-	-
	4	Heavy clay	1	M60
Kaliobia	8	Clay	1	K70
	3	Sandy clay loam	-	-
	7	Loamy clay	1	K73
Giza	5	Loamy clay	-	-
Dakahlia	9	Clay	2	d89, 90
Damietta	7	Clay	2	D96, 97
Alexandria	10	Sandy clay loam	2	A101, 107
Total	122		22	

only if a distance clear zone appeared in the spotted area, against the background lawn of the bacteria (**Dhar and Ramkrishna, 1987**).

Isolation of lytic phages

Phages in soil, which can infect *R. leguminosarum* bv. *viceae* were isolated by enrichment of soil samples from the rhizosphere of broad bean field in Sharkia Governorate, using the method described by **Patel and Graig (1984)**. The corresponding phages were diluted and assayed by double layer technique. **Adams (1959)**. Only, two lytic phages were obtained.

Purification of phage isolates

Phage isolates were purified by five successive single passage according to the method described by **Adams (1959)**. The final single plaque isolates were

designated as LP plus the number of their lysogenic host for temperate phages, and LP plus the number of soil sample for lytic phages. The lysate of each phage was centrifugated at 4000 rpm for 20 min, and the supernatent was filtered through Millipore membrane filter (0.45 μm). The filtrate had 10^7 to 10^8 plaques forming units/ml. The phages were stored in YEMB containing 0.5% chloroform at 4°C until use.

Characterization of rhizobiophags

Only, six out of 13 isolates of rhizobiophages, 4 temperate phages (TP_{27}, TP_{88}, TP_{97} and TP_{101}) and two lytic phages (LP_2 and LP_{11}) were chosen, according to their maintenance and titer. Some growth parameters for these selected phages were determined as follows:

Electron microscopy studies

One hundred milliliter of rhizobiophages suspension were centrifuged at 30000 rpm for 90 min, using Sigma 3 k 30 centrifuge, as recommended by Brenner and Horne (1959). The sediment was suspended in 15 ml of 1.0% ammonium acetate solution and recentrifuged at 5000 rpm for 15 min. A drop of the purified rhizobiophage suspension was applied to a 200-mesh copper grid coated with carbon. The grid was dried and the phage was negative stained with 20% uranyl acetate (pH4.5). Photographs were taken with Transmission Electron Microscope. Specimens were examined in Jule Electron Microscope (Model Jule 100 CX) at Electron Microscope Unit, Zagazig University, Egypt.

Adsorption experiments

Filtered laysate of the tested phages was added to a log phage of their sensitive rhizobia culture, at multiplicity of infectivity 0.1 ml, according to the method reported by Stent (1963). Samples were withdrawn every 5 min during incubation at 37°C for 40 min periodically, diluted (100-fold) in ice cold YEMB containing 10% chloroform and centrifugated at 5000 rpm for 5 min. The supernatant was filtered through millipore filter (0.45 μm). Unadsorbed phage particles were assayed and the adsorption rate was determined.

One-step growth curves experiments

One-step growth curve of each phage was determined according to the method mentioned by Adams (1959). Sensitive rhizobia of the tested phages were grown to a density of approximately 2.0×10^{10} CFU/ml, and infected with phages at a multiplicity of infection of 0.1 ml, and incubated at 28°C for 18 hr. After 5 min., samples of 0.1 ml were withdrawn at different intervals and plated onto soft agar with an indicator isolate. The observed numbers of phages in suspension were then plotted against time, and the latent period, rise period, and burst size were determined as recommended by Adams (1959).

Factors affecting activity of rhizophiophages

Some environmental conditions affecting both isolates of lytic and temperate rhizobiphages (heating, UV-irradiation, pH, storage periods at low temperatures) were determined as follows: The concentrated filtered phage

suspension of the tested phages was diluted to approximately 10^9 PFU/ml in 0.01 M phosphate buffer (pH 7.0) for studying the effect of pH on their survival, and the effect of heat on their viability as recommended by **Dhar and Ramkrishna (1987)**, as well as the effect of UV-irradiation on their activity, and the effect of two storage temperature degrees on their stability as described by **Dhar** *et al* **(1978)** were studied.

RESULTS AND DISCUSSION

In this work, eleven temperate phage specific for *R. leguminosarum* bv. *viceae* were isolated from 22 rhizobial isolates, which isolated from nodules of *Vicia faba* plants grown in different locations in Egypt. Also, two lytic phages were isolated from the soil samples of the rhizosphere of broad bean plants, out of 22 soil samples tested.

Host range susceptibility

The host specificity of eleven isolates of temperate phages were examined with 22 rhizobial isolates. The obtained results showed a wide variations of all isolated phages, when they were screened on the 22 rhizobial isolates, Table (2). The temperate phage (TP_{101}) found to have a wide host range, representing the maximum host range of the rhizobial isolates. Five rhizobial isolates (S_{26}, I_{41}, M_{52}, M_{60} and A_{100}) were lysed by this phage. Thus, phage TP_{101} is considered as polyvalent phage according to the definition of **Adams (1959)** who stated that specific phage lyse only a single strain of rhizobia, while polyvalent phage lyse numerous rhizobia strains. On the other hand, the phages TP_{73}, TP_{96}, TP_{119} have a limited host range, which reacted only with one rhizobial isolate. Thus, the latter phages could be nominated as specific phages.

Table 2. Host range susceptibility of isolated temperate rhizobiophages on the chosen isolates of *R. leguminosarum* bv. *viceae*

Temperate phages	Susceptible rhizobial isolates									
	No.	PFU/ml	No.	PFU/ml	No.	PFU/ml	No.	PFU/ml	No.	PFU/ml
TP24	S23	2.1×10^2	141	3.1×10^4	140	6.1×10^4	d89	7.1×10^5	-	-
TP27	S22	6.2×10^3	A101	7.2×10^2	S112	3.1×10^5	-	-	-	-
TP41	S23	1.2×10^3	M52	6.2×10^3	M60	5.2×10^2	-	-	-	-
TP73	K70	1.0×10^2	-	-	-	-	-	-	-	-
TP88	S26	3.1×10^2	S36	9.1×10^3	S27	2.0×10^5	-	-	-	-
TP96	d89	1.0×10^3	-	-	-	-	-	-	-	-
TP97	A107	2.0×10^4	S119	1.0×10^2	-	-	-	-	-	-
TP101	S26	5.0×10^2	141	7.0×10^3	M52	2.0×10^2	M60	7.0×10^4	A100	1.0×10^3
TP110	S22	3.0×10^3	S26	2.0×10^5	S112	7.0×10^4	-	-	-	-
TP112	S23	1.0×10^2	141	7.0×10^4	D96	2.0×10^3	d90	1.0×10^3	-	-
TP119	S22	7.0×10^2	-	-	-	-	-	-	-	-

With regard to the rhizobial isolates S_{22}, S_{23}, S_{26}, L_{41} and S_{112}, they appeared to have the higher susceptibility, which reacted with three or more isolates of phages. On the other hand, the rhizobial isolates S_{24}, S_{36}, K_{70}, K_{73}, d_{88}, d_{90} and d_{97} appeared to have the lowest susceptibility, which reacted only with one isolate of phage. Lysis of rhizobial isolates by the tested phages was completed mostly after 24 hours of incubation. The results obtained are in a good agreement with those found by **Barnet** (1972) who studied the host range of 28 phages from *R. trifolii*. She reported that some phages showed a wide host range, while others were restricted to one or a few hosts. Similarly, **Radwan** (1994) found that phages RV_2, RV_3, RV_6 and RV_{11} exhibited wide host range, since they infected different isolates of *R. leguminosarum* bv. *viceae* hosts. In contrast, phage RV_4 had a very narrow host range lysing only one of the 20 isolates of rhizobia tested. Again, **Ali et al** (1998) mentioned that phages having host ranges across different genera of root nodule bacteria may be important in horizontal gene transfer in soil and should be further explored.

In a trial to explain the relationship between the characters of the rhizobial strains and phage susceptibility determining the host range, **Emam et al** (1983) stated that the ability of phage particles to lyse rhizobia strains depended upon the presence or absence of receptors for rhizobiophage adsorption. Also, **Kankila and Lindstrom** (1994) reported that the host range of all types of phages was inversely related to the level of susceptibility of phage DNA to restriction enzymes.

Electron micrographs

Four selected temperate phages and two lytic phages, out of 13 isolated rhizobiophages, were examined and their morphological types as revealed by electron microscopy were described as shown in Figure (1) as follows:

The results obtained show that all the characterized phages negatively stained with uranyl acetate had a phage-like morphology with non-contractile tail. The isolated temperate phages (TP_{27} and TP_{97}) appeared to be similar, to some extent, and the other two temperate phages (TP_{88} and TP_{101}) were similar also in their dimensions and morphology, as shown in Figure (1). These phages had a prolate icosahedral head capsids as compared to the known phage T_4 (*E. coli* phage), and had a short tails and a distal tails knobs, which were visible in the virion. These examined phages were similar, to great extent, to the RL_4 phage isolated from *R. leguminosarum* SU-391 by **Dhar et al** (1987). Also, **Werquin et al** (1989) reported that *R. meliloti* phages heads were icosahedral, as shown by the observation of capsids with hexagonal and pentagonal outlines. As for the isolated lytic phages, the LP_2 phage had a spherical head and short tail, while the LP_{11} phage had icosahedral head, Fig. (1). Also, LP_2 phage had a visible distal tail knob, while the LP_{11} phage had a non-visible distal tail knob, as shown in their virions. Again, these tested lytic phages had a short tail and were similar to group C phages (8 phages) isolated from field grown *Vicia faba* plants in Sharkia Governorate, and examined by **El-Didamony** (1995). From the obtained results and according to the classification of **Bradley** (1967) these phages could be classified as belonging to
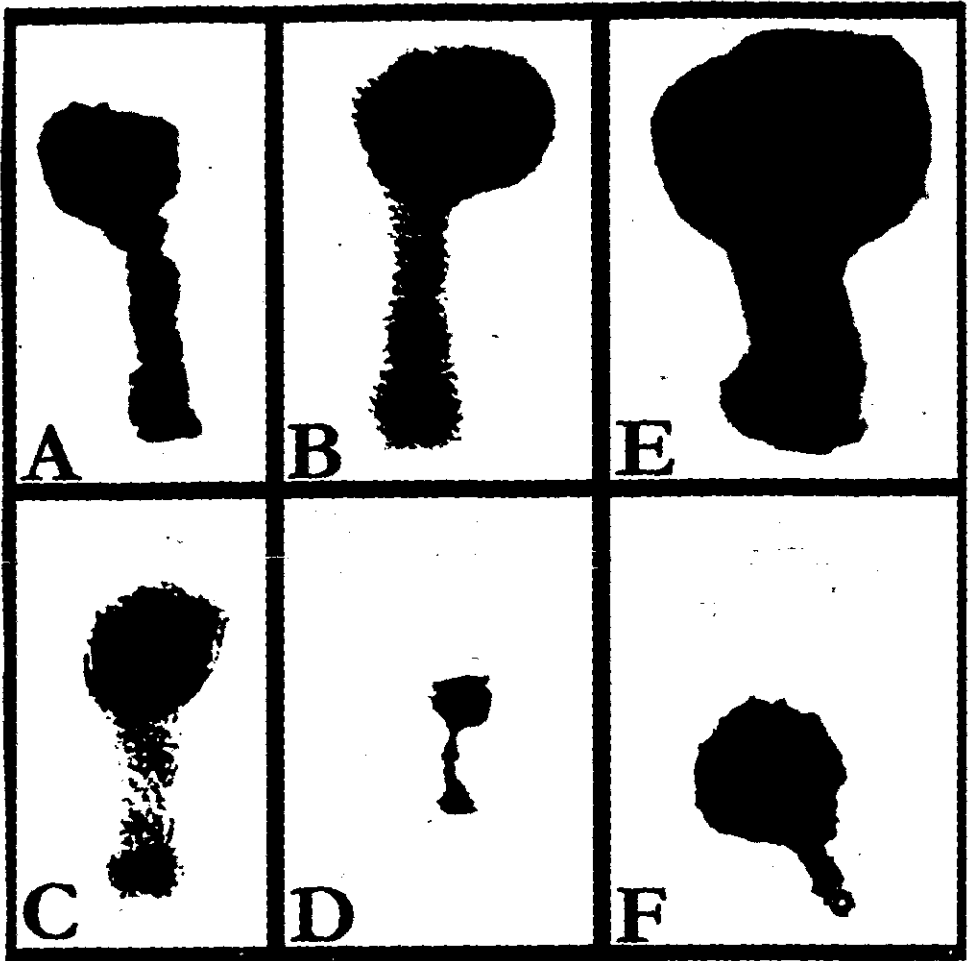
Figure 1. Electron micrographs of the tested temperate and lytic phage particles representing different morphological types

A: TP_{27} magnification, X 140000   B: TP_{88} magnification, X 40000
C: TP_{97} magnification, X 80000   D: TP_{101} magnification, X 80000
E: LP_2 magnification, X 140000   F: LP_{11} magnification, X 140000

group C, and they are supposed to contain double-stranded deoxyribonucleic acid as genetic material.

The results obtained show also the diversity of the tested phages that may be present in the rhizosphere and nodules of broad bean plants grown in different locations in Egypt. In this connection, **Sharma** *et al* (2002) suggested that rhizobiophages are maintained in the vicinity of their target host populations and are spread through the man-made introduction of host legumes and their rhizobia.

Adsorption rate

The tested six phages exhibited different adsorption rates, maximum adsorption times, and percentage of adsorption, Table (3). Adsorption rates were very slow and reached to their peaks after 25-30 minutes. The highest percentages of the adsorbed phage particles were 87.8, 85.7, 76.6, 79.0, 81.1 and 53.5% after 30, 30, 25, 30, 30 and 25 min for TP_{27}, TP_{88}, TP_{97}, TP_{101}, LP_2 and LP_{11}, respectively. Adsorption rates for temperate phages (TP_{27}, TP_{88} and TP_{101}) and LP_2 lytic phage revealed a linear curve up to 30 min., while phages TP_{97} and LP_{11} revealed a linear curve up to 25 min., which reached the maximum percentages of adsorption, then the adsorption rates were gradually decreased until the end of incubation period (40 min.). This might be a specific property of the phage-host system. These results are comparable very well with those reported by **Dhar** *et al* (1987) and **Radwan** (1994) who found that adsorption rates of rhizobiophages RV_2, RV_3 and RV_9 revealed a linear curve up to 30 min., which indicated that

adsorption rates followed a kinetics of the first order reaction.

One-step growth curve

It was determined for the tested six phages and the results are shown in Table (4). All the tested phages under study had almost identical latent periods (1 hour).

After that, there was an exponential rise in the phage titer, which reached the maximum after 5 hours for TP_{97}, LP_2 and LP_{11}, while it reached 6 hours for TP_{27}, TP_{88} and TP_{101} phages. In this concern, **Dhar** *et al* (1978) showed that the latent period of phage RL_1 specific *R. leguminosarum* was 90 min, and then the phage titer reached the maximum after 80 min. The average burst size, which were calculated from the PFU/ml values at the plateau of one-step growth curve, were 133, 365, 54, 38, 37 and 37 progeny phage per infected cell for TP_{27}, TP_{88}, TP_{97}, TP_{101}, LP_2 and LP_{11} phages, respectively. **Ley** *et al* (1972) found that the average burst size was 100 and 130 PFU/cells for RS_1 and RS_2 phages, respectively. Also, **Hashem** *et al* (1986) reported that burst size, rise period, and generation time for the phage active against *B. japonicum* USDA-117 were 100 PFU/cell, 12 min., and 80 min., respectively.

Some factors affecting rhizobiophages inactivation

Thermal inactivation

The sensitivity of the tested phages to heating for 20 min was studied, Figure (2). The temperate phages TP_{97} and TP_{101} were relatively more susceptible to heat (60, 65°C) than TP_{27} and TP_{88} phages.

Table 3. Adsorption rate of the tested temperate (TP) and lytic (LP) rhizophages for *R. legumuminosarum* bv. *viceae*

Incubation period (min.)	TP27		TP88		TP97	
	PFU/ml	Adsor. (%)	PFU/ml	Adsor. (%)	PFU/ml	Adsor. (%)
0	7.1×10^8	0.0	9.1×10^8	0.0	2.1×10^8	0.0
5	4.2×10^7	13.9	4.8×10^7	14.3	7.2×10^7	5.5
10	9.2×10^5	32.7	3.7×10^5	32.8	4.2×10^5	32.5
15	2.1×10^3	62.5	3.2×10^3	60.8	8.2×10^3	53.0
20	1.9×10^2	74.2	7.4×10^2	68.0	4.2×10^2	68.5
25	7.2×10	80.0	2.2×10^2	73.9	9.0×10	76.6
30	1.2×10	87.8	1.9×10	85.7	4.1×10^2	68.6
35	6.2×10^3	57.1	8.1×10^3	56.4	2.3×10^4	47.6
40	7.2×10^5	33.8	2.4×10^5	40.0	8.2×10^6	16.9

Table 3. Cont.

Incubation period (min.)	TP101		LP2		LP11	
	PFU/ml	Adsor. (%)	PFU/ml	Adsor. (%)	PFU/ml	Adsor. (%)
0	6.9×10^8	0.0	3.9×10^8	0.0	2.5×10^8	0.0
5	2.1×10^7	17.2	8.2×10^7	7.9	9.1×10^7	5.2
10	3.4×10^5	37.4	6.2×10^6	20.8	3.8×10^6	21.7
15	7.3×10^3	56.2	1.0×10^5	41.8	7.7×10^5	29.9
20	9.2×10^2	66.5	9.7×10^3	53.6	1.8×10^4	49.3
25	2.8×10^2	72.3	1.0×10^3	65.1	9.2×10^3	53.5
30	7.2×10	79.0	7.2×10	81.1	8.1×10^3	64.8
35	9.1×10^2	66.5	1.9×10^2	73.5	7.2×10^4	42.1
40	7.3×10^5	33.7	7.2×10^4	73.4	3.1×10^5	34.6

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Table 4. One-step growth curve of the tested temperature (TP) and lytic (LP) rhizobiophages for *R. legumuminosarum* bv. *viceae*

Incubation period (hr.)	TP27		TP88		TP97	
	PFU/ml	log	PFU/ml	log	PFU/ml	log
0	1.3×10^4	4.11	9.2×10^3	3.96	1.2×10^4	4.08
1	1.3×10^4	4.11	9.2×10^3	3.96	1.2×10^4	4.08
2	6.2×10^6	6.79	7.2×10^6	6.86	3.4×10^7	7.53
3	8.1×10^7	7.91	2.8×10^7	7.45	2.1×10^8	8.32
4	7.3×10^8	8.86	7.7×10^8	8.89	9.2×10^9	9.96
5	3.2×10^9	9.51	2.1×10^9	9.32	2.1×10^{10}	10.32
6	8.4×10^{10}	10.92	7.2×10^{10}	10.86	4.2×10^9	9.63
12	2.3×10^9	9.36	1.2×10^8	8.08	2.3×10^7	7.36
18	1.2×10^6	6.08	1.2×10^6	6.08	1.2×10^5	5.08

Table 4. cont.

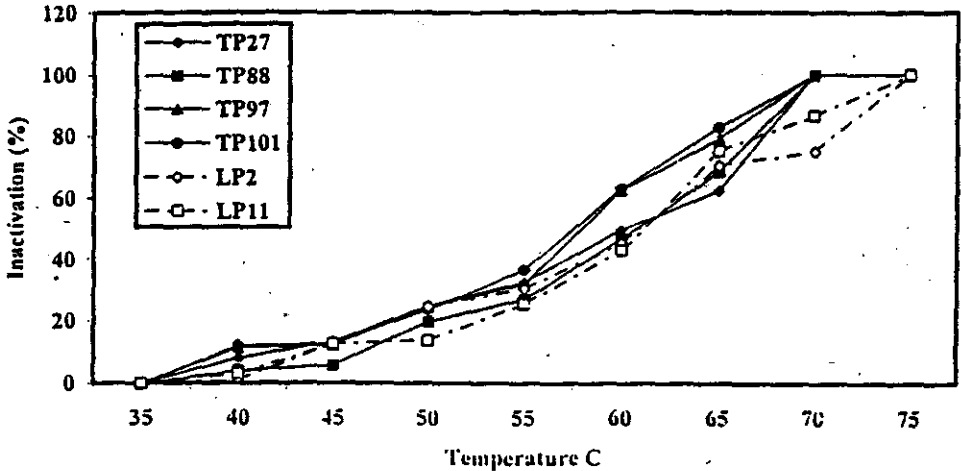| Incubation period (hr.)	TP101		LP2		LP11	
	PFU/ml	log	PFU/ml	log	PFU/ml	log
0	3.2×10^3	3.51	8.1×10^2	2.91	2.0×10^4	4.30
1	3.2×10^3	3.51	8.1×10^2	2.91	2.0×10^4	4.30
2	2.7×10^5	5.43	7.2×10^6	6.86	9.2×10^6	6.96
3	1.1×10^7	7.04	2.1×10^7	7.32	2.1×10^7	7.32
4	9.2×10^8	8.96	3.1×10^8	8.49	1.0×10^8	8.00
5	5.3×10^9	9.72	4.2×10^8	8.62	2.4×10^8	8.38
6	7.3×10^{10}	10.86	4.2×10^8	8.62	2.4×10^8	8.38
12	8.2×10^8	8.91	9.1×10^7	7.96	8.1×10^7	7.91
18	2.1×10^6	6.32	7.1×10^6	6.85	1.3×10^7	7.11

Figure 2. Inactivation percentage (%) of the tested temperate (TP) and lytic (LP) rhizo-
biophages by heating for 20 minutes

Their thermal inactivation patterns were exponential at 70°C. In lytic phages, the LP_{11} was relatively more susceptible to heat than LP_2 and their thermal inactivation patterns were exponential at 75°C. Generally, the activity of these phages decreased gradually when the phage particles were exposed to temperature more than 35°C for 20 min, and it was almost completely inactive at 70 to 75°C. Also, the temperate tested phages were generally more rapidly inactivated as compared to the tested lytic phages.

These results are in a good agreement with those of **Dhar** *et al* (1993) and **Ahmed and Morgan** (1994), since they found that inactivation of different rhizobiophages increased by increasing the temperature degrees. They also re-

ported that the percentage of inactivation varied and depended on the phage type and time of exposure and temperature degrees.

Sensitivity to UV-irradiation

It was determined for the tested phages and the results are recorded in Figure (3). In general, the phages were rapidly affected by increasing the time of exposure to UV-irradiation. Also, their activities were gradually decreased as the time of exposure was proceeded and they were completely inactive at 30 min. The TP_{88}, TP_{97} and LP_{11} phages were more sensitive to UV-irradiation than TP_{27}, TP_{101} and LP_2 phages, hence the first group lost their activities after 25 min.
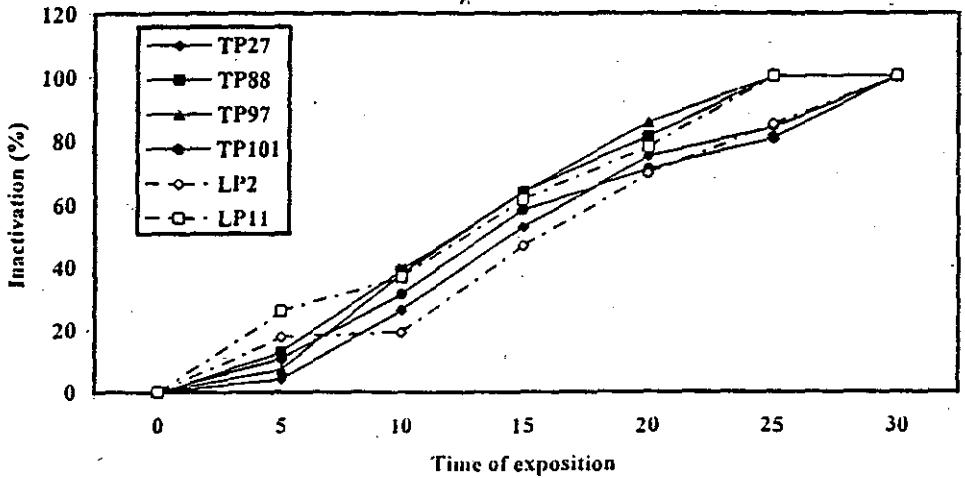
Figure 3. Inactivation percentage (%) of the tested temperate (TP) and lytic (LP) rhizo-
biophages by exposure to UV-irradiation

exposure, but the latter remained active after that and lost their activities completely after 30 min.

Based on the sensitivity of these phages, they could be classified into two groups, sensitive group (TP$_{27}$, TP$_{101}$ and LP$_2$) and relatively resistant ones (TP$_{88}$, TP$_{97}$ and LP$_{11}$). These phages seem to be more resistant to UV-irradiation as compared with those isolated by **Dhar and Ramkrishna (1987)** who found that RC$_1$, RC$_2$ and RC$_3$ were relatively resistant to UV-irradiation. However, the sensitivity of different rhizobiophages to UV-irradiation varied depending on phage type, time of exposure and distance, **Dhar et al (1993).**

Stability to different pH

The stability of the tested phages to different pH values was investigated in YEM medium, Figure (4). The results show that after incubation for an hour, the greatest stability was observed for those growing in the range of pH 7.0 to 8.0 for all the tested phages. The TP101 phage was the only isolate that tolerate a wide range of pH values, which ranged from less than pH 3.0 and more than 12.0, while LP$_2$ phage tolerate a narrow range which ranged from less than 4.0 and more than 9.0.

In general, the tested temperate phages were more stable than that of lytic
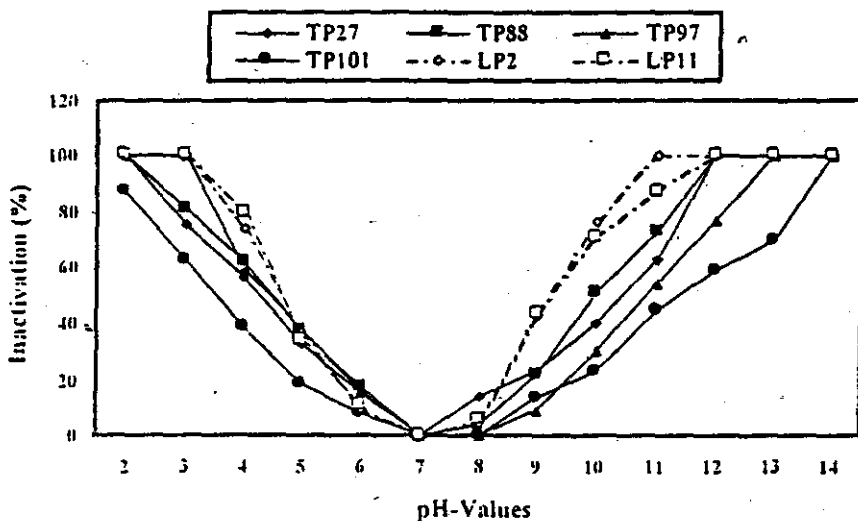
Figure 4. Inactivation percentage (5) of the tested temperate (TP) and lytic (LP) rhizo-
biophages at different pH values for one hour

phages. The survival of the former iso-
lates were different at pH values ranging
from pH 3.0 or less to pH 12.0 or more,
while the latter isolates were survived at
pH ranging from pH 4.0 to pH 10.0.
These results are in accordance with
those obtained by **Singh** *et al* **(1980)** and
Ahmed and Morgan (1994) who found
that the different rhizobiophages differ in
their stability at different pH values, and
they were more stable at pH 7.0. How-
ever, the inactivation % was increased on
both acidic and alkaline pH. Also, **Rad-
wan (1994)** mentioned that the greatest
stability was in the range of pH 6.0 to 8.0
for RV_2 phage and pH 6.0 to 10.0 for RV_3
phage of *R. leguminosarum* bv. *viceae* of
faba bean plants. He also stated that no
virus particles were detected at pH 3.0 or

at pH 12, while RV_3 phage tolerate a
wide range of pH, i.e., less than 3 and
more than pH 12.

Storage periods at low temperatures

The effect of storage periods (30, 60
and 90 days) at low temperatures (below
0 and 4°C) on the maintenance of the
tested phages are given in Figure (5). In
general, data show that increasing storage
time increased the percentage of inacti-
vation of all tested phages, progressively
by time. The rate of inhibition in the
phage titer was sharply increased during
storage periods at 4°C in comparison with
that in case of below 0°C (freezer) espe-
cially after 30 days of storage. These re-
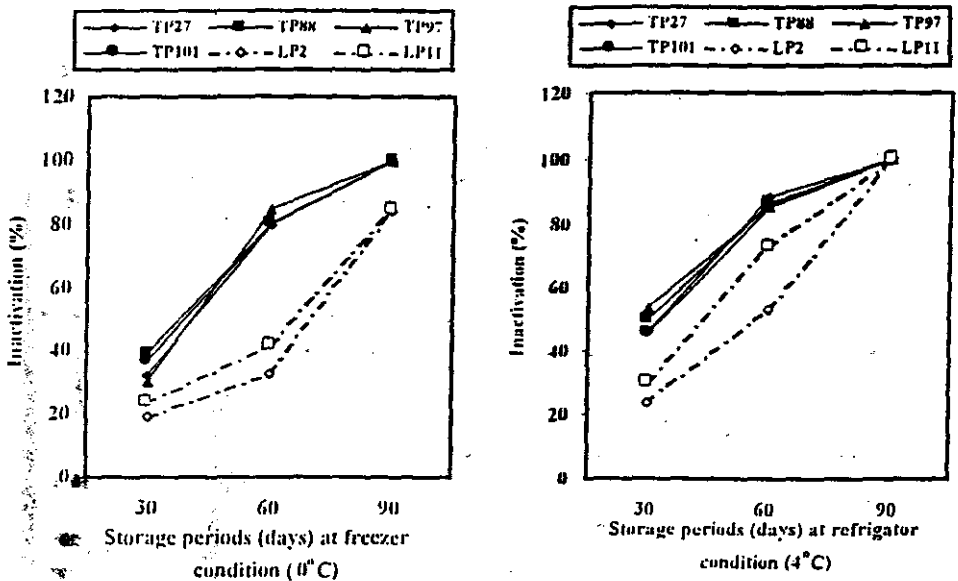sults are in a good agreement with those

Figure 5. Inactivation percentage (%) of the tested temperate (TP) and lytic (LP) rhizo-biophages by different storage periods at low temperatures (below 0 and 4°C)

of **Radwan** (1994) who reported that the optimum condition for the storage period of rhizobiophage was the maintenance below 0°C.

On the other hand, the results show that the lytic phages were more stable during storage period at low temperature (below 0°C) in comparison with the tested temperate phages. In addition, the lytic phages were more stable when they were stored in the freezer (below 0°C) for 90 days, while the temperate phages lost their infectivity after 60 days of storage period.

Similar results were recorded by **Agnes** *et al* (1989). They reported that the rhizobiophages isolated from cowpea remained infective for months when the phage was stored at a freezer condition.

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دراسة توصيفية لستة من البكتيروفاجات النشطة فى إصابة بكتيريا رايزوبيم ليجيومينيزارم بيوفار فياسى

[١]

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فى هذه الدراسة تم عزل وتنقية أربعـة من الفاجات الهادئة وأثنيـن مـن الفاجـات المحللة المتخصصين فى إصابـــة بكتيريـا رايزوبيم ليجيومينيزارم بيوفار فياسى وذلـك من عدد ٢٢ عينة للعقد الجذريـة وتربـة الرايزوسفير لنباتات الفول البلدى النامية فـى مناطق مختلفة من تسـعة محافظـات فـى مصر. وقد تم دراسة الشكل المورفولوجـى وبعض الخصائص البيولوجية لتلك الفاجـات المعزولة. وتأثير بعض العوامـل الطبيعيـة على نشاط وثبات تلك الفاجات.

وقد أظهـرت نتـائج فحـص المجـهـر الإلكترونى أن الفاجات الهادئة تحت الدراسة متماثلة من حيث الشـكل وهـى ذات رأس سداسية مكعبة الشكل وذيـل قصـير غـير منكمش وقاعدة محددة الذيل وذلـك بأبعـاد مختلفة ومن ناحية أخرى كــانت الفاجـات المحللة تحت الدراسة تنحصر فى مجموعتين مختلفتين مورفولوجيا فقد كان الفاج المحلـل LP₂ ذات رأس كروية وذيل قصـير غـير منكمش وقاعدة محددة للذيل بينما الفـاج LP₅ ذات رأس سداسية مكعبة الأبعاد وذيل قصير غير منكمش وقاعدة محـددة للذيـل غـير

مرئية. كما تشير نتائج دراسة عوامل النمـو لهذه الفاجات أنها تختلف اختلاف واضح فـى كل من معدل الأدمصاص – طور الصعـود – أقصى فترة صعود – منحنـى الخطـوة الواحدة – متوسط عدد الفاجات لكـل خليـة ولكن كانت تلك الفاجات ذات فترة حضانـة متماثلة (٦٠ دقيقة). وتظهر الدراسة أن هذه الفاجات ذات تفاعلات مختلفة عند دراسـة كل من الثبات الحرارى فى مدى مـن ٣٥م° إلى ٧٥م° ولمدة ٢٠ دقيقة – الثبـات عنـد تركيز أيون الأيدروجين فى مدى ما بيـن ٢ إلى ١٤ – الحيويـة عنـد التخزيـن علـى درجات الحرارة المنخفضة (درجة حـرارة الفريزر – درجة حرارة ٤م°) وذلك لمـدة – ٣٠، ٦٠، ٩٠ يوم من التخزين – الحساسـية عند التعريض للأشعة فوق البنفسجية لمسافة ٣٠سم ولمدة ٣٠ دقيقة. وبصفة عامة كـانت الفاجات المحللة (LP₁₁ ، LP₂) أكـثر ثباتـا وأعلى تركيزا من الفاجات الكامنـة تحـت الدراسـة (TP₂₋ ، TP₈₈ ، TP₉₇ – TP₁₀₁) وذلك عند دراسة العوامـل المؤثـرة علـى نموها.

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