FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (42), No.3, pp. 195 - 206 , 2022

BACTERIOPHAGES SPECIFIC TO A PHOSPHATE DISSOLVING BACTERIUM (BACILLUS *MEGATERIUM*)

I- ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES

Maha O. A. Omar
Dept. Agric. Microbiology- Fac. Agriculture – Minia University

Received : 28August 2022 Accepted: 30 September 2022

ABSTRACT

Ten isolates of Bacillus megaterium bacteriophages were isolated from a soil sample collected from the Experimental Farm of Faculty of Agric. Minia University. The isolated phages produced single plaques circular in shape, clear in appearance and their diameters ranged from 1 to 3 mm. The isolated bacteriophages were tolerant to pH 4 upto 12. pH 8 was the optimal for the isolated phages. The thermal inactivation point of six phages (Nos. 1,3,4,7, 8 and 9) was 85°C. Whereas, phages Nos. 2,5, 6 and 10 displayed different thermal inactivation point (95°C). Therefore, the ten phages were classified to two groups. Group (A) contained phages Nos. 1,3,4,7, 8 and 9 and group (B) included phages Nos. 2,5, 6 and 10. Sensitivity of phage isolates to U.V. radiation was studied. Phages of group (A) lost their infectivities after exposure for 60 min to U.V., whereas, phages of group (B) inactivated after 40 min. The host range of phages of each group was the same. The electron micrographs of the phages indicated that phages of each group were identical in sizes and morphologies. Therefore, the phages of each group were belonging to one phage type. i.e. the phages of group (A) were one phage type and designated Bm1 and phages of group (B) were another type and designated Bm2.

Key words: Bacteriophages, Bacillus megaterium, phosphate dissolving bacterium

INTRODUCTION

Bacteriophages are usually isolated from where the bacterial host is present. These viruses are of a particular interest, since they are likely have a negative effect in the ecology of their hosts specially those of economically importance in industrial and agricultural purposes. **Marie (2013)** isolated *B. subtlils* phages from 35 soil samples collected from different Egyptian Governorates.

Lysis of bacterial culture, morphology of plaque as well as morphology and size of particle and can

be used to characterize bacteriophages. Previous studies used combination of these techniques to characterize differences between phages.

Culture lysis has been used by many workers for detection of bacteriophages specific to different bacteria. Elsharouny (2014) detected the bacteriophages of Bacillus spp using the spot test. Moreover, Farahat (2016) detected the presence of bacteriophages of root nodule bacteria in Minia soils using the spot test. Such technique (spot test) is used just to indicate presence or absence of phages specific to particular bacteria, whereas, no more information about the isolated phages can be observed.

Plaque measurements and morphology are amongst the first feature which were used to classify phages of different bacteria **(Li and Zhang, 2014)**.

Elmaghraby *et al.* (2015) stated that the thermal inactivation poins of ten phages specific to *B. megaterium* were 78 and 82°C. Moreover, the thermal inactivation points of four *Bacillus subtilis* phages were found to be between 50-80°C **(Abo-Sinna 2004)**.

Elmaghraby *et al.* (2015) isolated ten phages of *Bacillus megaterium* tolerant to pH ranging from pH 5 to 9.

Efforts were made to characterize and identify bacteriophages specific to *Bacillus megaterium* using plaque morphology, thermal inactivation point, host range, tolerance to UV as well as morphology and size of particle. However, few number of phages was studied and the given details were limited,

Therefore, this study aimed to characterize the bacteriophages of B.megaterium which found in Minia soil according to plaque morphology, thermal inactivation point, sensitivity to U.V. light, the optimum pH of each phage isolate, host specificity as well as morphology and size of phage particles.

MATERIALS AND METHODS

Source of bacteriophages : Bacteriophages were isolated from a soil sample obtained from the Experimental Farm of Faculty of Agric. Minia University, Minia - Egypt.

The used bacteria: A bacterial isolate (*Bacillus megaterium*) efficient in dissolving phosphate and three *Bacillus* species (*B. cereus*, *B. subtilis* and *B. polymyxa*) were obtained from Dept. Agric. Microbiology, Fac. Agric. Minia University.

Isolation of Bacteriophages: Phages of *Bacillus megaterium* were isolated from the collected soil sample via liquid enrichment technique of **Adams (1966)** and **Barnet (1972).**

a- Phage Detection: phages of *Bacillus megaterium* were detected using the spot test according to **Adams (1966).**

b- Purification of bacteriophage isolates: Isolates of phages were purified according to **Kiraly *et al.* (1970)** using single plaque isolation technique.

c- Preparation of high titer phage suspension: High titer phage suspensions of the isolated phages were prepared using agar double layer plates method of **Maniatis *et al.* (1982**) as explained by **Hammad and Dora (1993)** and **Farahat (2016).**

d- Titer Estimation: Titer was determined as plaque forming unit (pfu)/ml according to **Kiraly *et al.* (1970)**.

4- Characterization of bacteriophages

a- The optimum: Single plaque of each phage isolate was placed in Eppendorf tube including 1m of SM medium with different pH levels (pH 4 upto 12) and incubated for 60 min. at 30°C. Ten µl from each tube was spotted on double agar layer plates (three replicates), seeded with *Bacillus megaterium* and incubated for 24 h at 30°C. Diameters of the lysed spots were measured (mm.) and the means of the three replicate was estimated.

b- Thermal stability: One ml of phage suspension of each single phage was heated in water baths at 50, 55, 60, 65, 70, 75, 80, 85, 90 and 95°C for 10 min, followed by cooling under tap water. The heated phage suspensions were spotted on double agar layer plates seeded with *Bacillus megaterium*. Plates were observed for lysed spots after 24 h. incubation at 30-33°C.

c- Stability to UV radiation: Petri dishes each containing five ml of high titer phage suspension of each phage isolate were placed at 20 cm away from UV lamp of 254 nm wave length. From each exposed phage suspensions to UV 10 µl was spotted on double agar layer plates seeded with *Bacillus megaterium*, after exposure to UV for 10, 20, 30, 40, up to 90 min. After incubation at 30°C for 24 h. Plates were observed for lysed spots.

d- Host specificity: Host specificity of each phage was estimated via the spot test. Each isolate of *Bacillus megaterium* phages was tested against the available *Bacillus* species.

e- Electron microscope examination: The isolated phages were observed by transmission electron microscope (Joel, Model GEM 1010) at 50 kv, according to **Hayat and Miller (1990)**. 0.5% uranyl acetate pH 4.5 was used to stain grids **(Stacey *et al*., 1984).**

REULTS AND DISCUSSION

Bacteriophages of *Bacillus megaterium*:

Bacteriophages specific to phosphate dissolving bacterium (*B. megaterium*) were enriched from a soil sample obtained from the Experimental Farm of Faculty of Agric. Minia University. Bacteriophages were detected using the spot test. **Figure (1)** showed that bacteriophages of *Bacillus megaterium* are common in the location of collecting the soil sample. Since it is assumed that phages are of widespread occurrence in areas which contain their bacterial host, presence of phages of *Bacillus megaterium*, may indicate the predominance of this bacterium in the locations from where samples had been collected. Such finding coincides with that found by **Hammad (1998 & 1999)** and **Fathy (2004)** who detected phages of *Azospirillum* and *Bacillus megaterium* in Minia soils.

Fig. (1): Petri plate seeded with *B. megaterium* and spotted with a drop of the phage lysate.

Purification of bacteriophages:

It is well known that each individual plaque has formed by the progeny of a single phage particle **(Kiraly, *et al.*, 1970)**. Therefore, the single plaque isolation technique was used to purify phages **(Figure 2)**. Plaque measurements and morphology are amongst the first feature that used to differentiate phages of different bacteria. The shape, size and outline of the plaques are characteristic of the phage strain **(Hammad, 1989).** Therefore, ten single plaques of *B. megaterium* phages each exhibited different morphology were picked and maintained as single isolate. The isolated plaques were circular in shape, clear in appearance and their diameters ranged from 1 to 3 mm. **Duff and Wyss (1961)** isolated and classified phages of *Azotobacter* depending upon the plaque morphology. **Barnet (1972)** stated that *Rhizobium trifolii* phages of the same particle morphology were found to have similar plaque characteristics.

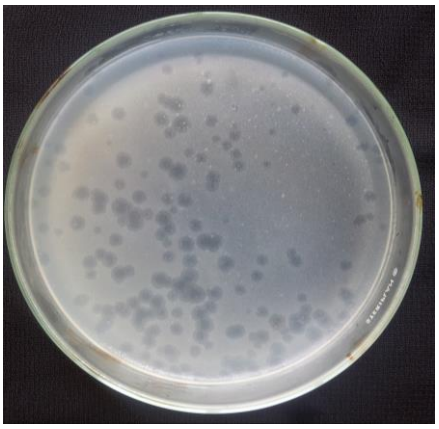

Figure (2): Individual plaques of phages specific to *B. megaterium* with different morphologies

Based on the information mentioned above, because of the ten single phage isolates of phages were different in their plaque morphology, it was expected that each phage isolate represents a single phage type. *i.e.* the phage isolates might be 10 different phage types of *B. megaterium.* In order to assess this expectation, the isolated phages were subjected to further characterization.

Titer of the prepared phage suspensions:

High titer phage suspension was prepared (two hundred ml for each isolate) to be used in this study. The obtained results as shown in **Table (1)** revealed that the titres ranged from 4.2 $x10^{10}$ pfu/ml to 9.7 $x10^{10}$ pfu/ml for phages of *B. megaterium*. Such high concentrations of phages was expected, since it is well known that a single

plaque of 2mm in diameter may contain between 10^7 and 10^9 recoverable phage particles **(Gunsalus and Stanier, 1960).**

Characterization of the phage isolates:

The different features of the ten isolates of phages were studied to know if these phages are similar or different types.

Determination of the optimum pH:

The infectivity of the ten isolates of phages was studied at pH 4 upto 12 . Data in **Table (2)** indicated that at any pH level tested, all phage isolates formed lysed spots. Such results indicate that the isolated bacteriophages of *B.megaterium* are tolerant to wide range of pH. Similarly stability of phages to different pH levels ranged from pH 5 to 12 was reported by **Fathy(2010); Roslycky *et al.* (1962); Challaghan *et al.* (1969); Hammad and Ali (1999).**

At pH 8 all phage formed lysed spots wider than those formed at any other pH tested. This may indicate that pH 8 is the optimum pH for all phage isolates.

On the basis of the obtained results different expectations are likely:

1) Since the optimum pH for all phage isolates is the same (pH 8) it is possible for the 10 phage isolates to be one phage type.
2) It is likely for the phage isolates to be different phage types and all having similar optimum pH.

The similarity of optimum pH for all phage isolates could be attributed to any of the above hypotheses, but cannot be acceptable without more characterization. Therefore, further characterizations were conducted to dismiss or accept any of aforementioned hypotheses.

Thermal stability of the isolated phages

Many investigators used the thermal inactivation point of the bacteriophages as a characteristic of bacteriophage isolates. **Hegazi *et al.* (1980)** reported that two isolates of *Azotobacter* bacteriophages were completely inactivated when exposed to 70°C and 80°C for 15 min. In addition, The different phage types of *B. japonicum* were found to have different thermal inactivation points (**Hammad, 1993** & **Hammad and Ali, 1999)**. Moreover, **Abo-Sinna (2004)** stated that the thermal inactivation points for 4 phages of *Bacillus subtilis* ranged between 50 - 80°C.

Elsharouny (2014) stated that phages of *Bacillus licheniformis* of single phage type showed the same thermal inactivation point, **Fathy (2010)** Stated that the phage isolates of *B. japonicum* and those of *R. meliloti* inactivated after incubation for 10 min. at 95°C and the thermal inactivation point of *R. phaseoli* is 75°C. **Marie (2013)** showed that the thermal inactivation point of *B. subtilis* phages is 68°C.

The results in **Table (3)** indicated that phage isolates of *B. megaterium* Nos. 1,3,4,7,8 and 9 have the same thermal inactivation point (85°C). Whereas, phage isolates Nos. 2,5, 6 and 10 exhibited different thermal inactivation point (95°C).

Therefore, due to the similarity in the thermal inactivation points the ten isolated phages were classified in two groups. Group (A) included phages No. 1,3,4,7,8 and 9 and group (B) comprised the isolated phages No. 2,5, 6 and 10.

These results can be explained in light of the following hypotheses:

1) The tent phage isolates of *B. megaterium* are likely belonging to two phage types according to their thermal inactivation points.
2) Since the ten phage isolates of *B. megaterium* under study were of different plaque morphologies. The phages of each group may be belonging to more than one phage type but exhibited the same thermal inactivation point.

Therefore, to accept one of these hypotheses further studies are needed.

Sensitivity to ultraviolet irradiation

Sensitivity of the isolated phages of *B. megaterium* to UV (254 nm) was tested. The obtained results in **Table (4)** indicated that the U.V. radiation at wavelength of 260 nm inactivated the isolated phages after different exposure times. Accordingly, the isolated phages of *B. megaterium* under study were divided to two groups. Each group included the phages which inactivated after similar exposure time.

Interestingly, the two groups of phage isolates which divided on the basis of the thermal stability **(Table 3)** were similar to as those divided based on the sensitivity to U.V.

The obtained results may indicate that the phages of each group are belonging to a one type of phages. This is just an expectation and additional characterizations are needed to be confirmed.

Host range assay:

Each of the ten phage isolates of *B. megaterium* was tested against four *Bacillus* species (*B. megaterium*, *B. cereus*, *B. subtilis* and *B. polymyxa*)

As shown in **Table (5)** two different host specificity for *B. megaterium* phages were detected for the ten phage isolates.

Accordingly, the phage isolates were classified in two groups (A and B). Each group included number of phage isolates, which exhibited similar host specificity.

Moreover, none of the ten phage isolates was specific but all were polyvalent phages, since, all phage isolates were infectious to more than one *Bacillus* species. **Barnet (1972)** stated that the ability of phage particle to lyse a bacterial strain is dependent upon the presence or absence of surface receptors for bacteriophages adsorption.

The two groups of *B. megaterium* phages, which divided on the basis of host range were found to be the same as those classified according to thermal inactivation points and sensitivity to U.V. radiation. These results may indicate that the phages of each group are one type of phages. To confirm these results morphology and size of phage particles were studied.

Size and morphology of phage particles:

The electron micrographs indicated that the ten phage isolates are of head and contractile tail types. According to the classification of the **International Committee on Taxonomy of Viruses (ICTV)** these phages were found to be belonging to Order *Caudovirales*, Family *Myoviridae*. The electron micrograph in **Figure (3 a)** represent the phages of group (A), *i.e.* phages Nos. 1, 3, 4, 7, 8 and 9 which were found to be identical in their morphologies, they possessed long

contractile tails of 135±2 nm in length, 11±3 in width and icosahedral head of 60±3 nm in diameter **(Table 6).** In addition, phages No. 2, 5, 6 and 10 of group (B) were found to be the same in their dimensions, they possessed long contractile tails of 122 ±3 nm in length, 13±2 nm in width and icosahedral head of 65±3 nm in diameter **(Figure 3 b).**

Therefore, phages of group (A) were considered one phage type and designated Bm1 and phages of group (B) represent another phage type and designated Bm2.

a **b**

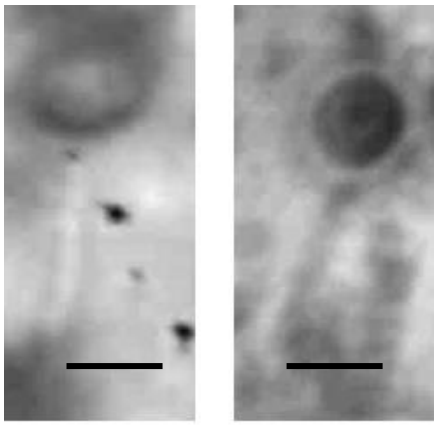

Figure (3): Electron micrographs of the isolated two phage types **(a)** Bm1 and **(b)** Bm2, negatively stained with uranyl acetate. Magnification bar = 50 nm.

Finally, on the basis of the above mentioned results, in this study different characteristics were used all together to classify the phage isolates of *B.megaterium*. No single technique for characterizing phages is in itself sufficient for classification, but these characteristics (optimum pH, thermal stability, sensitivity to U.V., host specificity as well as morphology and size of phage particles) must be studied all together to give clear differences between the phage isolates tested.

Table (1): Titer of the prepared phage suspensions

Phage No.	Titer (10^{10} pfu/ml)
1	**6.8**
2	**4.3**
3	**9.7**
4	**8.9**
5	**5.8**
6	**7.8**
7	**5.3**
8	**4.8**
9	**8.1**
10	**4.2**

The count of plaques represent the average of three replicates

Table (2): Stability of the ten phage isolates to different pH levels.

Phage No.	PH levels								
	4	5	6	7	8	9	10	11	12
	Diameter of the lysed spots (mm.)								
1	9.8	11.2	11.4	13.2	16.8	13.3	16.2	16.0	10.2
2	9.9	10.4	13.3	14.8	19.3	14.4	15.5	16.2	10.2
3	8.6	8.9	11.3	13.8	18.7	12.4	16.2	15.5	11.1
4	9.9	11.4	14.3	16.2	18.1	15.3	16.2	11.3	11.0
5	7.2	9.8	11.3	14.7	16.6	15.3	12.4	8.8	8.3
6	9.9	11.5	12.4	17.1	19.1	13.4	16.8	15.4	10.0
7	11.4	13.5	14.2	15.3	16.5	15.3	16.0	11.1	10.3
8	10.7	14.5	13.8	15.8	17.1	13.4	16.3	13.6	13.0
9	5.8	7.8	9.9	16.1	17.4	16.8	14.5	13.4	10.0
10	5.7	6.6	7.1	8.9	10.8	7.5	8.0	7.8	5.6

Diameters of the lysed spots represent the average of three replicates

The optimum pH.

Table (3): The thermal stabilty of B.megaterium phages.

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

+ = Lysis - = No lysis

Table (4): Sensitivity of phage isolates specific to *B. megaterium* to ultraviolet Irradiation

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

+ = Lysis **- = No lysis**

Table (5): The host specificity of ten phage isolates of *B. megaterium*.

Phage No.	*Bacillus* spp.			
	B. megaterium	*B. cereus*	*B. subtilis*	*B. polymyxa*
1	+	+	-	+
2	+	-	+	-
3	+	+	-	+
4	+	+	-	+
5	+	-	+	-
6	+	-	+	-
7	+	+	-	+
8	+	+	-	+
9	+	+	-	+
10	+	-	+	-

+ = Lysis **- = No lysis**

Table (6): Dimensions of *B. megaterium* bacteriophage particles.

Phage type	Tail		Head diameter ± SD (nm)
	Length ± SD (nm)	Width ± SD (nm)	
Bm1	135 ± 2	11± 3	60 ± 3
Bm2	122 ± 3	13 ± 2	65 ± 3

SD = Standard deviation

REFERENCES

Abo-Sinna, A. S. M. (2004). Studies on some viruses occurred under wheat cultivations in some Egyptian soils. Ph.D. Thesis, Faculty of science, Al-Azhar University, Cairo, Egypt.

Adams, M. H. (1966). The bacteriophages. Inter science Publishers. Inc., New York, pp. 447-461.

Barnet, Y. M. (1972). Bacteriophages of *Rhizobium trifolii*. I. Morphology and host rang. J. Gen. Virol., 15: 1-15.

Callaghan, R. E.; Mara W. O. and Grogan, J. B. (1969). Physical stability and biological and physiochemical properties of twelve *Pseudomonas aeroginosa* bacteriophages. Virology, 37: 642-649.

Duff, J. T. and Wyss (1961). Isolation and Classification of a new series of *Azotobacter* bacteriophages. J. Gen. Microbial. 24 : 273- 289.

Elmaghraby, I. ; Carimi, F.; Sharaf, A. Marei, E.M. and Hammad, A.M.M. (2015). Isolation and Identification of *Bacillus megaterium* Bacteriophages via AFLP Technique. Current Research in Bacteriology 8 (4): 77-89.

Elsharouny, T. H. M. (2014). Studies on bacteriophages specific for phosphate dissolving bacteria in soil. Ph. D. Thesis. Dept. Agric. Microbiol. Fac. Agric. Minia University, Egypt.

Farahat, E. M. M. (2016). Studies on some factors affecting growth and survival of root nodule bacteria of leguminous plants. M. Sc. Thesis. Botany dept., Faculty of Science, Beni-Suef Univ., Egypt.

Fathy, S. H. (2004). Protection of certain nitrogen fixing and phosphate dissolving bacteria against bacteriophage attack. M. Sc. Thesis, Dept. Agric.Microbiology, Fac. Agric. Minia Univ. Egypt.

Fathy, S. H. (2010). Studies on some factors affecting certain root nodule bacteria. Ph. D. Thesis, Dept. Agric. Microbiology, Fac. Agric. Minia Univ. Egypt.

Gunsalus, I. C. and Stanier, R. Y. (1960). The bacteria, A Treatise On Stracture and Function. Volume 1: Stracture. Academic Press, New York and London.

Hammad, A. M. M., (1989). A comparative study of bacteriophages of *Rhizobium leguminosarum* in soils of Egypt and Scotland. PhD Thesis, Fac. Agric., Minia University, Egypt.

Hammad, A. M. M. (1993). Occurrence of bacteriophages of *Bradyrhizobium japonicum* in rhizospher soil of soybean. Minia J. Agric. Res &Dev., 15: 609-624.

Hammad, A. M. M. (1998). Evaluation of alginate-encapsulated *Azotobacter chroococcm* as a phage-resistant and an effective inoculum. J. Basic Microbiol., 38 (1): 9-16.

Hammad, A. M. M. (1999). Induction of bacteriophage-resistant mutants of nitrogen fixing and phosphate dissolving bacteria. Annals Agric. Sci. Ain Shams Univ., Cairo, 44 (2): 479-493.

Hammad, A. M. M. and Ali, F. S. (1999). Bacteriophages of *Bradyrhizobium japonicum* in rhizosphere soil and their effect on

nodulation of soybean. Annals Agric. Sci. Ain-Shams Univ., Cairo 44 (1): 1-4.

Hammad, A. M. M. and Dora, S. A. (1993). DNA restriction patterns of *Bradyrhizobium japonicum* bacteriophages and their stability to U.V. radiation. Minia. J. Agric. Res. & Dev., 15: 591-608.

Hayat, M. A .and Miller, S. E. (1990). Negative Staining. McGraw-Hill Publishing Co.

Hegazi, N. A.; Abou-el-Nasr, M. A.; Othman, B. A. and Allam, E. K. (1980). Ecological studies on bacteriophages specific for a number of soil bacteria with particular reference to *Azotobacter*. Egyptian Society of Applied Microbiology. Proc. IV th Conf. Microbiol., Cairo 1980 Vol. 1: Soil,Food and Industerial Microbiol., pp. 283-302.

Kiraly, Z.; Klement, Z.; Solymosy, F. and Voros, J. (1970). Methods in Plant Pathology. With Special Reference to Breeding for Disease Resistance. pp 183-192, 2nd ed , Akademiai kiado, Budapest.

Li, L. and Zhang, Z. (2014). Isolation and characterization of a virulent bacteriophage *SPW* specific for Staphylococcus aureus isolated from bovine mastitis of lactating dairy cattle. Mol Biol Rep DOI 10.1007/s11033-014-3457-2.

Maniatis, T.; Fritsch E. F. and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual. New York: Cold Spring Harbor Laboratory.

Marie, E. M. (2013). Isolation and characterization of *Bacillus subtilis* phage from soil cultivated with Liquorices root. International Journal of Microbiological Research 4 (1): 43-49.

Roslycky, E. B.; Allen, O. N. and McCoy, E. (1962). Physiochemical properties of phages for *Agrobacterium radiobacter*. Canad. J. Microbiol., 9: 199-209.

Stacey, G.; Pocratsky, L. A. and Puvanesarajah, V. (1984). Bacteriophage that can distinguish between wild type rhizobium japonicum and a non-nodulating mutant. Applied and Environmental Microbiol., 48: 68-72.

.

الملخص العربي

الفيروسات البكتيرية المتخصصة على بكتيريا مذيبة للفوسفات (*Bacillus megaterium*)
- عزل وتوصيف الفيروسات البكتيرية

مها عمر عبداللطيف عمر
قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة المنيا

تم عزل عشرة عزلات من الفيروسات البكتيرية المتخصصة على بكتيريا Bacillus megaterium من عينة تربة تم جمعها من من مزرعة كلية الزراعة جامعة المنيا. أظهرت الفيروسات المعزولة مناطق تحلل منفردة (Plaques) دائرية الشكل وشفافة فى مظهرها وذات أقطار تراوحت بين 1 الى 3 مليمتر. تحملت الفيروسات المعزولة الظروف القلوية والحامضية حيث تحملت مدى واسع من الاس الهيدروجينى pH من 2 الى 12 . بينما كان الاس الهيدروجينى الأمثل لحدوث الاصابة هو pH 8 . درجة التثبيط الحرارى لعزلات الفيروسات رقم 1، 3، 4، 7، 8 و 9 بلغت 85°م بينما العزلات الفيروسية ارقام 2، 5، 6 و 10 بلغت درجة التثبيط الحرارى لها 95°م . ولذلك تم تقسيم الفيروسات المعزولة الى مجموعتين ، مجموعة (أ) تضم العزلات أرقام ، 3، 4، 7، 8 و 9 اما مجموعة (ب) تشتمل على العزلات أرقام 2، 5، 6 و 10 . تم دراسة حساسية الفيروسات المعزولة للأشعة فوق البنفسجية حيث تم تثبيط فيروسات المجموعة (أ) بعد تعرضها للأشعة لمدة 60 دقيقة, بينما تم تثبيط فبروسات المجموعة (ب) بعد التعرض للاشعة لمدة 40 دقيقة. تم دراسة المدى العوائلى للفيروسات المعزولة حيث تبين ان فيروسات كل مجموعة ذات مدى عوائلى واحد. بدراسة أشكال وأبعاد الجزيئات الفيروسية تبين أن عزلات كل مجموعة ذات أشكال وأبعاد متطابقة . من هذه النتائج تبين ان فيروسات كل مجموعة تنتمى الى نوع واحد من الفيروسات. اى ان العشرة عزلات الفيروسية تنتمى الى نوعين فقط من الفيروسات البكتيرية. وقد تم تسمية فيروسات المجموعة (أ) Bm1 وفيروسات المجموعة (ب) Bm2 .