

## Coliphages Lysing *Escherichia coli* Bacteria: Their Morphology and Their Response to Some Physical Factors

<sup>1</sup>Gamal Enan, <sup>2</sup>Khalid A. Shaaban, <sup>1</sup>Ahmed Askora and <sup>1</sup>Muhammad Maher

<sup>1</sup>Department of Botany and Microbiology, Faculty of Science, Zagazig University, Zagazig, Egypt

<sup>2</sup>Soils, Water and Environment Research Institute, Agriculture Research Center, Cairo, Egypt

**Abstract:** Three coliphages designated ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> were identified in this study. Also, the effect of some physical factors on the infectivity of these coliphages was studied. The three coliphages were tested for their infectivity using the indicator bacterial strains *E. coli* W<sub>1</sub> and *E. coli* W<sub>2</sub> where coliphage ECP<sub>1</sub> was highly specific to infect *E. coli* W<sub>1</sub>, on the other hand, coliphages ECP<sub>2</sub> and ECP<sub>3</sub> were highly specific to infect *E. coli* W<sub>2</sub>. The three coliphages were very sensitive to heat inactivation no survivors could be recorded after 10 min of exposure time up to 60°C. Also, the profile of the three coliphages inactivation after different time intervals exposed to UV-irradiation at different heights of 15 and 30 cm from the irradiation source was examined. Increased UV-irradiation doses decreased the plaque numbers of the three coliphages tested. The effect of different pH values on stability of the three coliphages was also tested. Maximum stability was observed at pH 6.0. The viabilities of the three coliphages were rapidly decreased towards alkalinity compared to pH 6.0. The ability of the three coliphages to form plaques at preservative temperature range 3-5, 25-28 and 35-37°C was examined. The suitable storage temperatures for the three coliphages were 3-5 and 25-28°C.

**Key words:** *E. coli*, coliphages, sewage water, raw water, temperature, UV-irradiation, pH value, storage

---

### INTRODUCTION

Bacteriophages (phages) are viruses which infect bacteria. Coliphages are phages that infect *E. coli* bacteria. Lytic phages can provide a natural and nontoxic method for detecting and controlling the growth of human pathogens (Ackermann and DuBow, 1987; Enan *et al.*, 2012b; Abdallah *et al.*, 2013). Coliphages infecting *E. coli* bacteria were used in many previous studies as biological indicators of fecal contamination of water (Grabow *et al.*, 1998). Coliphages are taxonomically very diverse, covering six virus families viz. Myoviridae, Styloviridae, Podoviridae, Microviridae, Inoviridae and Leviviridae (Colford *et al.*, 2007).

The use of coliphages as biological indicators of microbial contamination faces some limitations concerning difficulty of detection methods using agar plates in analyzing larger volumes of water. In addition coliphages or other phages did not denote on all bacterial types of water (Love and Sobsey, 2007). Consequently, it is better to use phages to control pathogens in water such as the use of coliphage to control growth of *E. coli* in water (Levy *et al.*, 1988). It is also recommended to use phages as biotherapy (Sulakvelidze *et al.*, 2001). Search on safe biotherapy is of interest and there is a need to concentrate

scientific research on this point, because previous researchers recommended the use of phage in control of some human pathogens (Chanishvili *et al.*, 2001) and also in control of veterinary pathogens (Matsuzaki *et al.*, 2003; Enan *et al.*, 2012b; Abdallah *et al.*, 2013). The present research was undertaken to characterize three coliphages infecting *E. coli* W<sub>1</sub> and *E. coli* W<sub>2</sub> bacteria all were isolated from water. This is to start research about control of *E. coli* strains in water by their coliphages.

### MATERIALS AND METHODS

**Isolation of bacterial strains and coliphages:** HiCrom *E. coli* selective agar media (HiCrom™) were used for isolation of the experimental *E. coli* W<sub>1</sub> and *E. coli* W<sub>2</sub> strains. Two *E. coli* strains were isolated from polluted raw water that was collected from the River Nile in Eastern part of Egypt. Those two strains were designated *E. coli* W<sub>1</sub> and *E. coli* W<sub>2</sub> and were used as an indicator bacterial strains for isolation and propagation of coliphages.

Coliphages infecting *E. coli* W<sub>1</sub> and *E. coli* W<sub>2</sub> strains were isolated from sewage water samples of sewage plant of Bahr El bakar canal in Western part of Egypt. Sewage water samples were filtered through membrane filters (0.45µm Millipore, Amicon) to get water

free bacteria. The filtrate was inoculated with the indicator *E. coli* W<sub>1</sub> and *E. coli* W<sub>2</sub> strains and was further inoculated with an equal volume of nutrient broth (Oxoid) for 24 h. This mixture was centrifuged at 3000 rpm for 30 min. After removing precipitates, the mixture was passed through membrane filter (0.45 µm Millipore, Amicon) to remove heavy metals of high molecular mass. The filtered supernatants were then filtered and assayed for phage activity by double layer agar technique using soft nutrient agar (Adams, 1959; Enan *et al.*, 2012b; Abdallah *et al.*, 2013). Plaques of coliphages were picked up and propagated in plates to give confluent lysis. To attain coliphages with high titers, plates developed obvious plaques were washed and their fluid of washes were treated with chloroform at final concentrations 0.5% (Jensen *et al.*, 1998; Enan *et al.*, 2012b; Abdallah *et al.*, 2013).

**Identification of indicator bacteria:** The criteria reported by Enan *et al.* (2013) were used for identification of bacteria at the genus level. Consequently, Gram staining, cell morphology, motility, catalase reaction, oxidase test, production of H<sub>2</sub>S and growth on HiCrom media confirmed that the W<sub>1</sub> and W<sub>2</sub> strains belong to genus *Escherichia*. The species was determined via API 20 kits as reported by the manufacturer's instructions (Biomereux, France). Identification was carried out according to Garrity *et al.* (2005) and Enan *et al.* (2012a, 2013).

**Electron microscopy study:** Specimens were prepared for electron microscopic examination from partially purified coliphage or 50 mL filtered coliphage suspension (from confluent lysis plates) which was precipitated at 20,000 rpm of ice cooling centrifuge at 4°C for 24 h (Adams, 1959). Drops of high titer filtered coliphage suspension were laid on formavar coated copper grid (400 meshes) with carbon coated colloidal membrane and then negatively stained by uranyl acetate (4% aqueous). Excess fluids were withdrawn by a filter paper strip (Monod *et al.*, 1997). Specimens were washed 3 times by distilled water, dried by filter paper or air dried and viewed by electron microscopy. Morphological assessment of the isolated coliphages was performed by transmission electron microscopy (JEOL JEM.1010) at 80 kV and magnification range of 50X-500KX (Electron microscopy unit, Faculty of Medicine, Zagazig University, Egypt).

**Effect of temperature on the infectivity of the three coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub>:** The 2 mL of each coliphage suspension were incubated singly at 30, 35, 40, 45, 50, 55 and 60°C for 10 min (Dhar and Ramkrishna, 1987). Phage infectivity was determined by plaque assay technique (Adams, 1959).

**Effect of UV-irradiation on the infectivity of the three coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub>:** Sensitivity to UV-irradiation was determined by exposing 10 mL of coliphage suspension, separately, in a petri dish directly to UV light at distances of 15 or 30 cm from the UV source (240 nm). A 30 W general electric lamp was used as UV source. All manipulation was made in the dark to avoid photoreaction. Samples of phage suspensions were exposed separately to UV-irradiation for different time periods (30, 60, 90 to 180 min) and the samples were assayed by double layer technique (Dhar and Ramkrishna, 1987).

**Effect of pH on the infectivity of the three coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub>:** Coliphage suspensions were incubated at 37°C for 30 min in nutrient broth at pH values ranging from 4-10 and the survived coliphage was determined by double layer technique by using *E. coli* W<sub>1</sub> and *E. coli* W<sub>2</sub> bacteria as an indicator strains.

**Effect of different storage temperatures on the coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> infectivity:** Coliphage suspensions of high titer lysates were incubated in 3-5, 25-28 and 35-37°C separately for 5 weeks. The infectivity of coliphages was assayed weekly by double layer technique as described above (Dhar and Ramkrishna, 1987; Abdallah *et al.*, 2013).

## RESULTS

**Isolation and identification of bacteria:** Representatives of colonies growing on HiCrom media (HiCrom™) were picked up and purified on the same media. All the bacterial isolates were rod shaped, motile, Gram negative and catalase positive cells. Based on the manufacturer's instructions of API 20 Streps, all bacterial isolates showed positive results with regard to indole test, methyl red test, utilization of glucose, lactose, maltose, mannitol, L-arabinose, D-sorbitol but showed negative results Regarding Coagulase test, Oxidase test, Voges Proskauer test, citrate utilization, H<sub>2</sub>S production and utilization of sucrose and salicin.

Following diagnostic key of Garrity *et al.* (2005), all the *Escherichia* isolates were identified and classified as belonging to *E. coli*. Two strains were selected according to their sensitivity to coliphages and designated *E. coli* W<sub>1</sub> and *E. coli* W<sub>2</sub>.

**Isolation and purification of coliphages:** Three coliphages were isolated from sewage water using the spot test and double layer method of plaques assay (Fig. 1). Based on plaques appearance, three coliphages were chosen. Coliphage<sub>1</sub> (ECP<sub>1</sub>) showed small clear circular plaques but

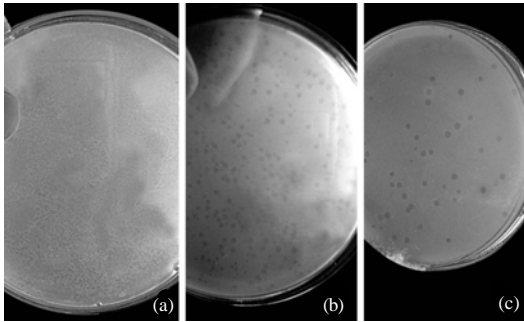


Fig. 1: Plaques morphology of the isolated coliphages after 24 h of incubation; a) plaques of coliphage ECP<sub>1</sub> showing small clear circular plaques; b) plaques of coliphage ECP<sub>2</sub> showing moderate clear circular plaques and c) plaques of coliphage ECP<sub>3</sub> showing large clear circular plaques

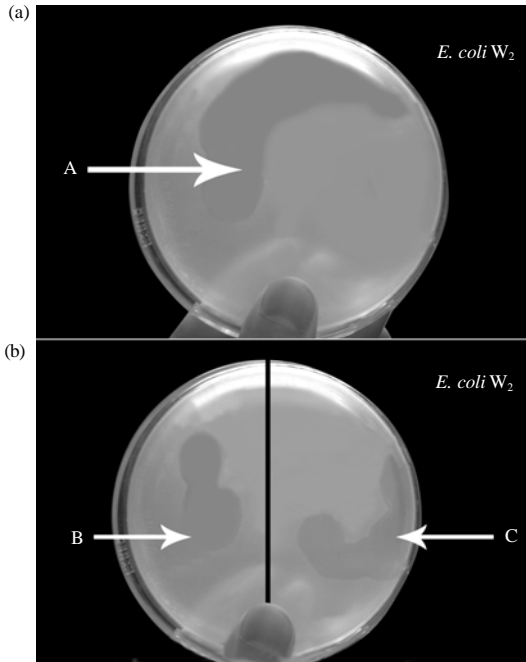


Fig. 2: a, b) Effect of spotting of the isolated coliphages on the isolated bacterial strains (*E. coli* W<sub>1</sub> and *E. coli* W<sub>2</sub>); A) ECP<sub>1</sub>; B) ECP<sub>2</sub> and C) ECP<sub>3</sub>

coliphage<sub>2</sub>, coliphage<sub>3</sub> (ECP<sub>2</sub>, ECP<sub>3</sub>) showed moderate, large clear circular plaques respectively (Fig. 1). ECP<sub>1</sub>, ECP<sub>2</sub>, ECP<sub>3</sub> were propagated and purified for further study. A large clear zones have been developed after spotting of purified coliphage on lawn of bacterial indicator by 14 h (Fig. 2).

**Morphology of coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub>:** Transmission electron microscopy was employed to

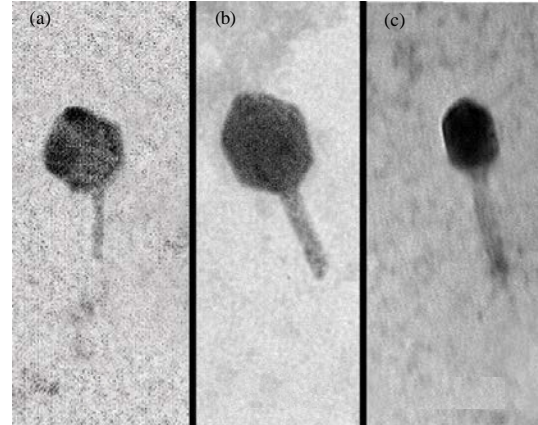


Fig. 3: Electron microscopy of the isolated coliphages; a) ECP<sub>1</sub>; b) ECP<sub>2</sub> and c) ECP<sub>3</sub>

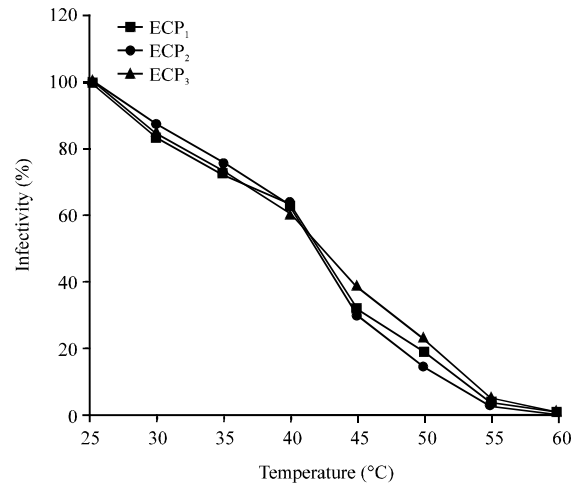


Fig. 4: Effect of temperature on the infectivity of the three coliphages

observe the morphology of coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> (Fig. 3). The coliphages ECP<sub>1</sub> and ECP<sub>2</sub> had isometric hexagonal head (50×40 and 90×70 nm, respectively) while coliphage ECP<sub>3</sub> possessed elongated hexagonal head (120×100 nm). The three coliphages had contractile tails and their lengths were 40×10, 80×10 and 200×40 nm for ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub>, respectively (Fig. 3). Consequently, the coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> were classified as belonging to family Myoviridae (group A) (Bradley, 1967; Mathews, 1982).

**Effect of temperature on the infectivity of the three coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub>:** Coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> were highly sensitive to heat inactivation (Fig. 4). No survivors could be recorded after exposure of these coliphages to 60°C. The highest numbers of plaques were observed at 30°C, reaching 83.2, 87.2 and 84.1% for

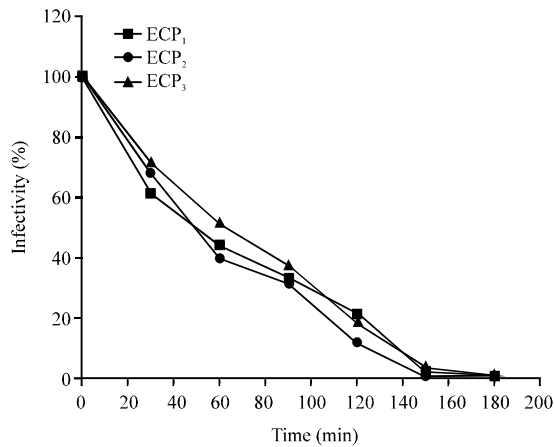


Fig. 5: Inhibition of the three coliphages by exposing to UV-irradiation (at a distance of 15 cm from the UV source)

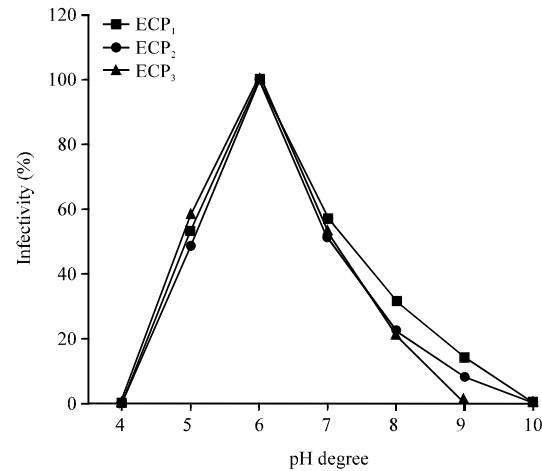


Fig. 7: Effect of pH value on the infectivity of the three coliphages

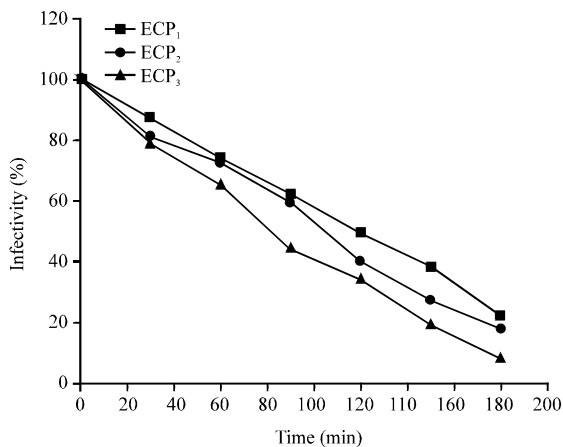


Fig. 6: Inhibition of the three coliphages by exposing to UV-irradiation (at a distance of 30 cm from the UV source)

the three coliphages, respectively. The viability of the three coliphages was decreased with increasing the temperature.

**Effect of UV-irradiation on the infectivity of the three experimental coliphages:** The exposure of coliphages to the UV-irradiation had inhibitory effect on the coliphage infectivity and the increasing of the exposure time caused decreasing in the coliphage infectivity (Fig. 5 and 6).

At 15 cm height from UV source, a 180 min exposure was sufficient for complete inactivation of coliphages. In addition, ECP<sub>2</sub> was inactivated completely at 150 min. Therefore, ECP<sub>2</sub> was the most sensitive to UV-irradiation than ECP<sub>1</sub> and ECP<sub>3</sub>.

At 30 cm height from UV source, a 180 min exposure was not sufficient for complete inactivation of coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub>. A 77.9, 81.9 and 91.6% inactivation were observed after 180 min of exposure to UV-irradiation for coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub>, respectively. Surprisingly no change in plaque morphology was observed after UV-irradiation under the studied conditions.

**Effect of pH on the infectivity of the three coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub>:** The results shown in Fig. 7 showed that the optimum pH values for the three coliphages were determined by incubation of coliphage suspensions at different pH values ranging from 4-10 for 30 min. Then the remaining coliphages particles were survived by double layer technique. As observed in Fig. 7, the three coliphages were very sensitive to hydrogen ion concentrations. The optimum pH 6.0 was the best pH value for the coliphages stability. The viability of the three coliphages was rapidly decreased towards alkalinity compared to pH 6.0. Moreover, the plaques formation of the coliphage ECP<sub>3</sub> was more sensitive to hydrogen ions exchange than coliphages ECP<sub>1</sub> and ECP<sub>2</sub>.

**Effect of different storage temperatures on the coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> infectivity:** The ability of ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> to form plaques at different storage temperatures was determined using *E. coli* W<sub>1</sub> and *E. coli* W<sub>2</sub> as an indicator strains by double layer technique. The 3-5 and 25-28°C temperatures were suitable for coliphage infectivity while the 35-37°C temperature was suitable for coliphage infectivity till the 5th week for ECP<sub>3</sub>.

Table 1: Effect of different storage temperatures on coliphages (ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub>) infectivity

Temperature	Time (weeks)	ECP <sub>1</sub>		ECP <sub>2</sub>		ECP <sub>3</sub>	
		PFU/mL ×10 <sup>7</sup>	Percentage	PFU/mL ×10 <sup>7</sup>	Percentage	PFU/mL ×10 <sup>7</sup>	Percentage
Control	0	2.99	100.0	4.06	100.0	4.93	100.0
3-5°C	1	2.78	93.2	3.88	95.6	4.78	97.1
	2	2.45	82.1	3.54	87.4	4.50	91.4
	3	2.23	74.6	2.96	73.1	4.05	82.3
	4	1.82	61.2	2.63	64.8	3.67	74.6
	5	1.44	48.4	2.12	52.3	3.25	66.1
25-28°C	1	2.66	89.1	3.72	91.7	4.61	93.7
	2	2.29	76.8	3.22	79.5	4.19	85.1
	3	1.77	59.2	2.37	58.4	3.26	66.2
	4	1.31	44.1	1.63	40.2	2.67	54.3
	5	0.78	26.1	1.26	31.1	2.11	42.9
35-37°C	1	2.52	84.3	3.55	87.6	4.50	91.4
	2	1.98	66.5	2.63	64.9	3.76	76.4
	3	1.17	39.2	1.37	33.8	2.81	57.1
	4	0.49	16.4	0.53	13.2	1.62	32.9
	5	0.00	0.0	0.00	0.0	0.97	19.7

and the 4th week to ECP<sub>1</sub> and ECP<sub>2</sub>. So, ECP<sub>1</sub> and ECP<sub>2</sub> were more sensitive to the 35-37°C temperature than ECP<sub>3</sub> as shown in Table 1.

### DISCUSSION

The growth of bacterial isolates on HiCrom medium and other biochemical, morphological and cultural criteria facilitated the identification of *E. coli* strains isolated from water (Garrity *et al.*, 2005; Enan *et al.*, 2012a, 2013). Three coliphages named ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> were isolated from sewage water. ECP<sub>1</sub> was specific for *E. coli* W<sub>1</sub> while the other ECP<sub>2</sub> and ECP<sub>3</sub> were specific for *E. coli* W<sub>2</sub>. According to the morphological features appeared by electron microscopy and based on taxonomic criteria reported by Bradley (1967) and Mathews (1982), the three coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> were classified as belonging to group A of family Myoviridae.

Thermal inactivation pattern showed that the three coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> were highly sensitive to elevated temperature and the numbers of plaque forming units were greatly reduced. The obtained results revealed that the three coliphages were inactivated after exposure to 60°C for 10 min. These results were confirmed by Kuroda and Bradley (1968) who mentioned that three phages named SAP1, SAP2 and SAP3 were inactivated by exposure to 60°C for 1 h. They found that the replication of these phages were least affected by 40°C. El-Tarabily *et al.* (1995) found that the phage numbers ΦS1, ΦS2 and ΦS3 specified to *Streptomyces diastaticus*, *Streptomyces griseus* and *Streptomyces hygroscopicus*, respectively were markedly decreased by exposing to 45 or 56°C for 15 min. The infectivity of the three coliphages gradually decreased with increasing the time and decreasing distance of exposure to UV-irradiation till the complete inhibition taken place. The three coliphages

were completely inhibited after exposure to this irradiation at a distance of 15 cm for 150 min (coliphage ECP<sub>2</sub>) or 180 min (coliphages ECP<sub>1</sub> and ECP<sub>3</sub>). On the contrast the coliphages were still infective after exposure to UV-irradiation at 30 cm for 180 min. Therefore, these coliphages were resistant to exposure to UV-irradiation and similar results were published by Dhar and Ramkrishna (1987) and El-Helali (2001).

Stability of the three coliphages of current study at different pH values was investigated. The three coliphages were very sensitive to change in the hydrogen ions concentration with an optimum value of pH 6.0. The viability of these coliphages was rapidly decreased towards alkalinity. At pH 10.0, plaques formation were not observed for ECP<sub>1</sub> and ECP<sub>2</sub> while at pH 9.0, plaques formation were not observed for ECP<sub>3</sub>. Therefore, the respective percentages of survivors at pH 5.0 were 53% for ECP<sub>1</sub>, 48% for ECP<sub>2</sub> and 58% for ECP<sub>3</sub> compared to counts at pH 6.0. At pH 7.0, 8.0 or 9.0, the respective percentage of survivors reached 56, 31 and 14% for ECP<sub>1</sub> or 51, 22 and 8% for ECP<sub>2</sub> or 53, 20 and 0% for ECP<sub>3</sub>, respectively compared to counts at pH 6.0. Delbruck (1950) revealed that pH value of the suspending medium was an important factor affecting stability of phages and similar results in this respect were published by Jang and Kim (1996). Sykes *et al.* (1981) found that a wide range (5.5-9.0) of pH values were valuable for the stability of phages virulent on acidophilic *Streptomyces*. The optimum pH value for the *Streptomyces griseus* phages were generally between 7.0 and 8.0 these values have, therefore been used for the satisfactory storage of the phage for at least 2 years (Change, 1953).

On the other hand, Rautenshtein *et al.* (1975) reported that the particles of temperate phage infecting *Streptomyces rimosus*, a producer of oxytetracyclin, lost lytic activity by incubation at pH 4.0 for 15 min.

## CONCLUSION

The viability of the three coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> in different storage temperatures were found to be more stable at 3-5 or 25-28°C than 35-37°C. The infectivity of the three coliphages was lost with prolonged incubation time. Similar results were published in this respect (El-Tarabily *et al.*, 1995; Patten *et al.*, 1995). Further research will be necessary regarding complete physicochemical and biological characters of the coliphages employed herein. This will be important to start research about use of the coliphages ECP<sub>1</sub>, ECP<sub>2</sub> and ECP<sub>3</sub> for inhibition of *E. coli* *in vitro* and in water.

## REFERENCES

- Abdallah, F.M., H. Sobhy and G. Enan, 2013. Evaluation of antiviral activity of selected anise oil as an essential oil against bovine herpes virus type-1 *in vitro*. *Global Vet.*, 10: 496-499.
- Ackermann, H.W. and M.S. DuBow, 1987. Viruses of Prokaryotes, Volume II: Natural Groups of Bacteriophages. CRC Press Inc., Boca Raton, FL., USA., ISBN-13: 9780849360572, Pages: 256.
- Adams, M.H., 1959. Bacteriophages. Interscience Publishers Inc., New York.
- Bradley, D.E., 1967. Ultrastructure of bacteriophage and bacteriocins. *Bacteriol. Rev.*, 31: 230-314.
- Change, C.H., 1953. Studies on phages with special reference to host specificity. Ph.D. Thesis, University of Wisconsin.
- Chanishvili, N., T. Chanishvili, M. Tediashvili and P.A. Barrow, 2001. Phages and their application against drug-resistant bacteria. *J. Chem. Technol. Biotechnol.*, 76: 689-699.
- Colford Jr., J.M., T.J. Wade, K.C. Schiff, C.C. Wright and J.F. Griffith *et al.*, 2007. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. *Epidemiology*, 18: 27-35.
- Delbruck, M., 1950. Viruses 1950: Proceedings of a Conference on the Similarities and Dissimilarities between Viruses Attacking Animals, Plants and Bacteria, Respectively. California Institute of Technology, Pasadena, CA., USA., pp: 100-147.
- Dhar, B. and K. Ramkrishna, 1987. Morphology and general characteristics of phages of chickpea rhizobia. *Arch. Microbiol.*, 147: 121-125.
- El-Helali, M.F.M., 2001. Studies on some biotic and environmental factors affecting the activity of *Rhizobium leguminosurum* biovar *viceae*. Ph.D. Thesis, Botany Department, Faculty of Science, Zagazig University, Egypt.
- El-Tarabily, K.A., D.I. Kurtboke and G.S.J. Hardy, 1995. Partial characterization of *Streptomyces* phages isolated from the soils of Jarrah forest in western Australia. *Actinomycetes*, 6: 7-15.
- Enan, G., F.M. Abdallah and H. Sobhy, 2012a. Effect of acyclovir on bovine herpesvirus type 1 infection in *in vitro* cultured cells. *Int. J. Virol.*, 8: 307-312.
- Enan, G., N. Awny, A.A. Abou Zeid and M.A. Abdou, 2012b. Incidence and virulence of *Bacillus cereus* isolated from Egyptian foods during four seasons. *Afr. J. Microbiol. Res.*, 6: 4816-4824.
- Enan, G., S. Abdel-Shafi, S.M. Ouda and I. El-Balat, 2013. Genetic linkage of the antibiotic resistance ability in the *Escherichia coli* UR<sub>4</sub> strain isolated from urine. *J. Med. Sci.*, 13: 261-268.
- Garrity, G., D.G. Brenner and N. Krieg, 2005. The Alpha, Beta, Delta and Epsilon Proteobacteria. In: *Bergey's Manual of Systematic Bacteriology*, Boone, D.R., D.G. Brenner, J.T. Staley, P. De Vos, G.M. Garrity, M. Goodfellow and N.R. Krieg (Eds.). 2nd Edn., Springer, New York, USA.
- Grabow, W.O.K., A. Very, M. Uys and J.C. De Villiers, 1998. Evaluation of the application of bacteriophages as indicators of water quality. WRC Report No 540/1/98, Water Research Commission, Pretoria, pp: 55.
- Jang, Y.H. and H.U. Kim, 1996. Effects of cultural conditions on the adsorption of *Lactobacillus casei* bacteriophages to the host cells. *Korean J. Dairy Sci.*, 18: 269-274.
- Jensen, E.C., H.S. Schrader, B. Rieland, T.L. Thompson, K.W. Lee, K.W. Nickerson and T.A. Kokjohn, 1998. Prevalence of broad-host-range lytic bacteriophages of *Sphaerotilus natans*, *Escherichia coli* and *Pseudomonas aeruginosa*. *Applied Environ. Microbiol.*, 64: 575-580.
- Kuroda, S. and S.G. Bradley, 1968. Characterization of polyvalent phages for *Streptomyces aureofaciens*. *Adv. Front. Plant Sci.*, 19: 29-42.
- Levy, S.B., B. Marshall, S. Schluederberg, D. Rowse and J. Davis, 1988. High frequency of antimicrobial resistance in human fecal flora. *Antimicrob. Agents Chemother.*, 31: 1801-1806.
- Love, D.C. and M.D. Sobsey, 2007. Simple and rapid F<sup>+</sup> coliphage culture, latex agglutination and typing assay to detect and source track fecal contamination. *Applied Environ. Microbiol.*, 73: 4110-4118.
- Mathews, R.E.F., 1982. Classification and Nomenclature of Viruses: Fourth Report of the International Committee on Taxonomy of Viruses. S. Karger, Basel, Switzerland, ISBN-13: 978380553557, Pages: 199.

- Matsuzaki, S., M. Yasuda, H. Nishikawa, M. Kuroda and T. Ujihara *et al.*, 2003. Experimental protection of mice against lethal *Staphylococcus aureus* infection by novel bacteriophage phi MR11. *J. Infect. Dis.*, 187: 613-624.
- Monod, C., F. Repoila, M. Kutateladze, F. Tetart and H.M. Kirsch, 1997. The genome of the pseudo T-even bacteriophages, a diverse group that resembles T4. *J. Mol. Biol.*, 267: 237-249.
- Patten, K.M., D.I. Kurtboke and D.R. Lindsay, 1995. Isolation of *Dermatophilus congolensis* phage from the Lumpy Wool of sheep in Western Australia. *Lett. Applied Microbiol.*, 20: 199-203.
- Rautenshtein, Y.I., V.V. Zhumaeva, Y.M. Khokhlova, N.G. Nesterova and L.N. Moskalenko, 1975. Factors causing lysis of an *Actinomyces rimosus* culture, the producer of the antibiotic, oxytetracycline. *Mikrobiol. Zh.*, 44: 899-904.
- Sulakvelidze, A., Z. Alavidze and J.G. Morris Jr., 2001. Bacteriophage therapy. *Antimicrob. Agents Chemother.*, 45: 649-659.
- Sykes, I.K., S. Lanning and S.T. Williams, 1981. The effect of pH on soil actinophages. *J. Gen. Microbiol.*, 122: 271-280.