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DETECTION OF *XANTHOMONAS VESICATORIA* PHAGES IN INFECTED TOMATO PLANTS

[28]

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ABSTRACT

Different Phages parasitizing ***Xanthomonas vesicatoria*** (the causal agent of the bacterial spot disease of tomato) were isolated from infected leaves of tomato and from tomato rhizosphere soil, using enrichment technique. The phages produced plaques (4-5mm, diameter) with a distinct translucent spreading halo. Presumptive phage particles associated with ***X. vesicatoria*** were observed by Transmission Electron Microscope (TEM). Particle size and morphology of each phage isolate were examined by electron microscopy. The obtained results indicated that, the isolated phages were of the head and tail types. Four phages were detected and designated A,B,C and D, their head diameters were found to be 57.83, 43.84, 63.43, and 79.25 nm, respectively. Phages A and B were isolated from tomato leaves, whereas, phages C and D were isolated from tomato rhizosphere soils. This study seems to be the first record for phages of ***Xanthomonas vesicatoria*** under the Egyptian conditions.

Keywords: Phage, *Xanthomonas vesicatoria*, Tomato, Bacterial spot disease, Enrichment technique.

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INTRODUCTION

Bacterial spot disease of tomato and pepper caused by *Xanthomonas vesicatoria* causes significant losses when warm temperatures and rainy weather occurs (**Jones *et al.*, 1991**).The disease is observed worldwide in tomato and pepper production areas (**Bouzar *et al.*, 1994**). Recently, the disease was observed in several tomato and pepper production areas in Egypt (**Abd El-Ghafar** and **Abd El-Wahab, 2001, Abd El-Ghafar** and **Mosa, 2001** and **El-Meneisy, 2005**).

Bacteriophages of plant pathogenic bacteria can be isolated from soil or from infected plant tissue. Phages isolated from soil are mostly polyvirulent, while phages isolated from infected tissues are highly specific (**Persley,1983**). Phages are very specific that they can be used to identify their bacterial hosts in certain cases (**Cuppels, 1984**). Host specificity is an ideal attribute as biocontrol agent (**Wall and Sanchez, 1992**).

Bacteriophages have been used in identification of bacteria, strain characterization, epidemiological studies and even in attempts which have been made in biological control studies (**Billing, 1969 and Persley, 1983**). Interaction of phages with phytopathogenic bacteria including their potential for disease control has been the subject of several review articles (**Okabe** and **Goto, 1963; Civerolo, 1972 and Vidaver, 1976**).

Several studies have shown that phages active against *Xanthomonas campestris* pathovars have a high degree of specificity (e.g. *Xanthomonas vesicatoria pv. pruni; pv. carotae and pv. vesicatoria*) (**Eisenstark and Bernstein, 1955 and Klement, 1959**). **Stolp and Starr (1964)** made a major study on the phages of Xanthomonas isolated from soil, but the phages did not show a useful degree of specificity. The Xanthomonas phages isolated from plant material were more specific.

The present work was carried out to study the occurrence of bacteriophages of *Xanthomonas vesicatoria* in infected tomato leaves and in tomato rhizosphere soil. In addition, plaque morphology as well as particle size and morphology of the isolated phages were also studied.

MATERIALS & METHODS

1. Bacterial isolates and inoculum preparation:

Three isolates of *Xanthomonas vesicatoria* were isolated from infected tomato plants. Bacterial isolates were grown on peptone sucrose agar (PSA)medium at 28°c for 48 hours and suspended in sterilized distilled water (SDW). Peptone sucrose broth (PSB) medium was inoculated with bacterial suspension and incubated at 28° c for 48 hours. Inoculated PSB medium was used to isolate and propagation of phage (**Myung *et al.*, 2002**).

2. Isolation and purification of phages:

Infected and /or healthy leaves of tomato and rhizospheric soils of tomato samples were collected from Kalubia Government. The enrichment culture technique was used for isolation of phages according to (**Eayre *et al.*, 1995**).

The infected leaves (spotted leaves) were crushed in crucible discernible and then added to 200 ml of the prepared liquid bacterial culture in 250 ml Erlenmeyer flask.

The rhizosphere soil samples were suspended in SDW plus sterilized tween80 (2 to 3 drops/flask) and were shaken in rotary shaker at 3000 rpm for 20min.then filtrated through a hydrophobic filter 0.2 um. The filtrates were added to 10 ml of sterilized 1% $CaCO_3$ and 5 ml of 48h.old liquid culture of *Xanthomonas vesicatoria* in 250 Erlenmeyer flask containing 200 ml of PSB medium.

Erlenmeyer flask prepared for either crushed leaves or for soil filtrates were shaken for 72 h. at 28 °C in a rotary shaker. The culture were centrifuged at 10000 rpm for 10 min. the agar double layer plates were routinely used for isolation of single plaques where plaques of the phages were purified by three cycles of single plaque isolation to obtain single purified plaques for phage lytic types. Purified phages were stored in 2 ml plastic vials at 4°C in complete darkness.

3. Electron Microscopy:

Scanning Electron Microscope (TEM) was used to examine phages of *Xanthomonas*

vesicatoria. This study was carried out at the electron microscopy laboratory of the Microbiology and Cell Science Department, Faculty of Science, Al Zaher University, Cairo, Egypt.

The phages were visualized using negative staining protocol with 1% aqueous uranyl acetate. Where, a drop of the phage suspension was added to a 300-mesh copper grid and the droplet was partially wicked off using a triangle- shaped piece of filter paper. The remaining thin layer of liquid was left on the grid.

After 3 minutes, 1%of uranyl acetate was added to the grid and droplet wicked off after 45 second. This procedure was repeated with distilled water and after immediate partial wicking of the water droplet, the grid was air-dried. The grids were examined by Scanning Electron Microscope (TEM). Images were made with image view 3 digital camera using analysis soft ware. **(Chen and Civerolo, 2008)**.

RESULTS & DISCUSSION

Phages of *Xanthomonas vesicatoria* were isolated from infected leaves of tomato and from tomato rhizosphere soil samples, using enrichment technique. These samples were collected from Kalubia governorate. The phages were mostly isolated from infected leaves of tomatoes and also from the rhizosphere soil.

The isolated bacteriophages formed single plaques of different morphological characteristics. The formed plaques were circular with irregular margins or without determined margins and ranged in their diameters form 4 to 5 mm.

Particle size and morphology of each phage isolate were examined by electron microscopy. As shown in **Figure (1) and Table (1)** the obtained results indicated that, the isolated phages were of the head and tail types. Four phage types were detected and designated A,B,C, and D, their head diameters were found to be 57.83, 43.84,٦٣،٤٣ , and 79.25 nm, respectively. Phages A and B were isolated from tomato leaves, whereas, phages C and D were isolated from tomato rhizosphere soils.

Ackermann (2007) summarized all of the known phages of *X. vesicatoria* infecting

tomato plant into four morphological groups (tailed, polyhedral, filamentous and pleomorphic) classified into 20 families using nucleic acid analysis and other properties. He observed phage- like particles in the tailed, polyhedral and pleomorphic morphological groups. However, based on morphology, the large icosahedral particles were placed in the podovridae but further proof of the presence of short tails is needed; the small icosahedral particles could be classified in the family Micrviridae, The tailed particles could be placed in the Siphoviridae, and the filamentous particles were placed in the family Inoviridae.

Phage infection is generally determined by attachment of phage to its bacterial host bacterium. The attachment to host cell is a primary factor for determining multiplicity of phages in cells , and is affected by several factors **(Garen and Kozloff, 1959)**.

Bacteriophages are capable to penetrate their host cells and multiplying within them, exploit the cell "component and biochemical pathway "to their own advantage. Phages which are very specific that they can be used to identify their bacterial hosts in certain cases (**Cuppels, 1984**).

Phages with a high degree of specificity have frequently been found in infected plant material **(Hayward, 1964 and Billing, 1963**). Highly specific phages have also been obtained from soil beneath infected plants **(Persley and Crosse, 1978).**

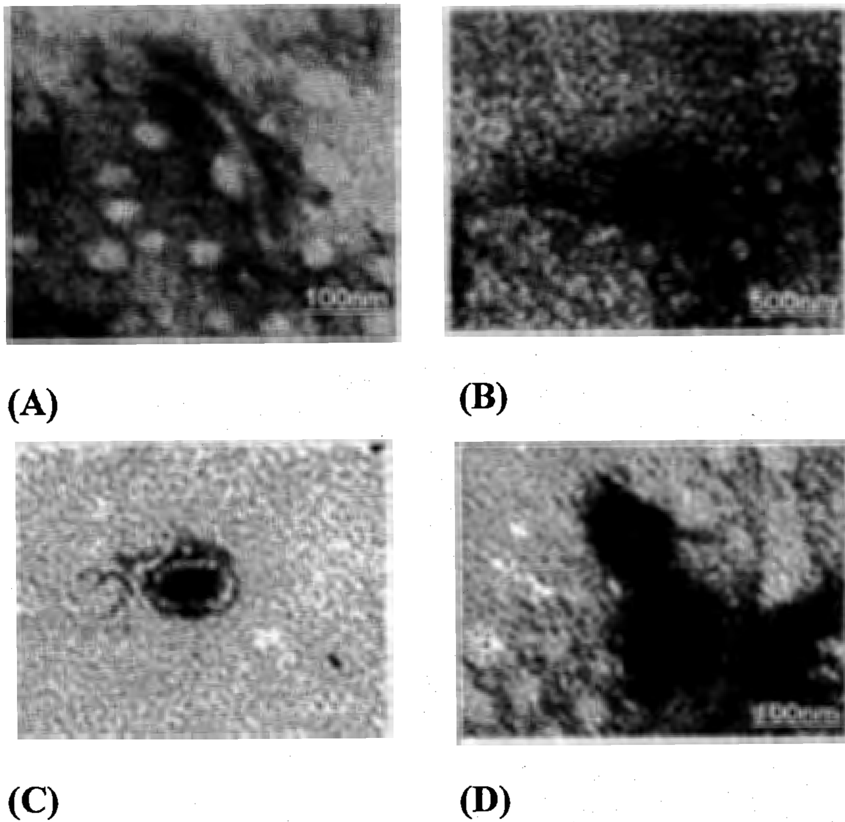

Figure 1. Electron micrographs of *X.vesicatoria* bacteriophages. Phages A and B were isolated from tomato leaves, phages C and D were isolated from tomato rhizosphere soils.

Table 1. Dimensions of *Xanthomonas vesicatoria* phages.

Phage Type	Head diameter (nm)	Tail length (nm)
A	57.83	29.6
B	43.84	22.74
C	٦٣,٤٣	25.63
D	79.25	33.71

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