THE DISTRIBUTION AND THE LYSIS PATTERNS OF THE BACTERIOPHAGES OF *XANTHOMONAS MALVACEARUM*, THE INCITANT OF BACTERIAL BLIGHT OF COTTON

by R. P. SINGH *and* J. P. VERMA, *Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi*-110012

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Bacteriophages, specific to *Xanthomonas malvacearum* (E. F. Smith) Dowson, the incitant of bacterial blight of cotton, were isolated from all the infected specimens of *Gossypium hirsutum* L., soil and water. Plaque size was largest on potato-sucrose-peptone-agar-medium, and best plaque formation was observed at 25–30°C. The thermal inactivation point was 75–80°C and phage titre 8×10^{10} per ml. Phages could be isolated from two years old diseased cotton tissues. Twenty-three phage isolates were collected and these could be classified into two distinct groups: group I (9 phages) lysing only the lactose non-utilising isolates of the pathogen and group II (14 phages) lysing only lactose *utilising isolates*.

INTRODUCTION

Bacteriophages, specific to plant pathogenic bacteria (Okabe and Goto 1963; Stolp and Starr 1964) including *Xanthomonas malvacearum* (Massey 1931) have been isolated and these have been utilised in various ways in taxonomy, detection and epiphytological studies. Singh *et al.* (1970) were the first to successfully isolate bacteriophages specific to *X. malvacearum* from India. The present paper is an attempt to describe the distribution of these bacteriophages in India, their lysotype and use in grouping Indian isolates of *X. malvacearum*.

MATERIALS AND METHODS

Bacteriophages in the infected plant parts (stem, leaf, boll, seed) and soil/water were enriched for 48 hr with fresh *Xanthomonas malvacearum* cultures, after which the phage was collected from the phage-enriched-broth-culture by centrifugation and filtration through bacteriological sintered glass filter. The final filtrate was tested for the presence of bacteriophage by spot test and plaque test methods. Single plaques were suspended in 2 ml sterilised distilled water columns and purified repeatedly till uniform plaques were obtained. These were finally picked up in 2 ml water columns and stored at 5°C. These were designated as standard stock phage.

Actively growing 24 hr cultures of *X. malvacearum* on PSPA–slants (potato-sucrose-peptone-agar-slants; potato, 300g; peptone, 2g; sucrose, 20g; KH_2PO_4, 0.2g; Na_2HPO_4, 0.5g; calcium nitrate, 0.5g; KCl, 0.05g; ferrous sulphate, 0.05g; agar 20g

per litre) were suspended in 10 ml distilled sterilised water and termed as standard bacterial suspension.

Twenty-three phages (XMP_1–XMP_{23}) were isolated from infected plant parts, soil and water.

RESULTS

Distribution of bacteriophages in India — Infected leaves and in one case soil were obtained from various locations and tested for the presence of bacteriophages. Results (Table I) showed that bacteriophages, although generally distributed in India could not be recovered from *Gossypium barbadense* of Junagadh and Surat; *G. arboreum* of Khandwa and *G. herbaceum* of Ludhiana. Presence of phages was shown in all other samples and the infested soil sample of Sirsa. A general conclusion appears to be that bacteriophages are present in heavily infected areas and in all the *G. hirsutum* material. It may be mentioned that Ludhiana *G. hirsutum* material was two years old, thereby showing that bacteriophages could be isolated from fresh as well as from at least two year old samples stored under dry conditions at room temperature.

TABLE I

Distribution of bacteriophages of Xanthomonas malvacearum *in India*

Locality		*Gossypium* species	Source	Presence (+) or absence (—)	Phage Number
State	Place				
Delhi	IARI	*G. barbadense* & *G. hirsutum*	Leaf, petiole, stem, boll, seed, water, soil	+	XMP-1 to XMP-12
Gujarat	Junagadh	*G. barbadense*	Leaf	—	—
		G. hirsutum	,,	+	XMP-13
,,	Surat	*G. barbadense*	,,	—	—
		G. hirsutum	,,	+	XMP-14
Uttar Pradesh	Bullundshahr	*G. barbadense*	,,	+	XMP-15
		G. hirsutum	,,	+	XMP-16
,,	Raya	*G. barbadense*	,,	+	XMP-17
		G. hirsutum	,,	+	XMP-18
Haryana	Sirsa	*G. hirsutum*	,,	+	XMP-19
			Soil	+	XMP-20
Punjab	Ludhiana	*G. hirsutum*	,,	+	XMP-21
		G. herbaceum	,,	—	—
Madhya Pradesh	Khandwa	*G. arboreum*	,,	—	—
		G. hirsutum	,,	+	XMP-22
,,	Indore	*G. hirsutum*	,,	+	XMP-23

TABLE II

Grouping of 23 phage isolates on the basis of lytic (+) or nonlytic (—) action on 20 isolates of Xanthomonas malvacearum

Phage isolate	Bacterial isolate number (XM—)																			
	Lactose non-utilising									Lactose utilising										
	47	50	57	74	75	91	107	114	121	24	51	58	60	61	92	93	106	112	126	132
XMP-1	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-2	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-3	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-4	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-5	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
XMP-6	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
XMP-7	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
XMP-8	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-9	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-10	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-11	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-12	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-13	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
XMP-14	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
XMP-15	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-16	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-17	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-18	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
XMP-19	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
XMP-20	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
XMP-21	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
XMP-22	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
XMP-23	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+

Grouping of phage isolates — All the 23 phage isolates were grouped on the basis of lysis to 20 isolates of the bacterium belonging to two biochemical groups i.e. group I [lactose non-utilising (lac—)] and group II [lactose utilising (lac+)]. Lysis was studied by spot test technique. The results (Table II) show that one group of 9 phages (XMP-5, 6, 7, 13, 14, 18, 19, 20 and 21) lysed lac— while another group of 14 phages (XMP-1, 2, 3, 4, 8, 9, 10, 11, 12, 15, 16, 17, 22 and 23) lysed only lac+ isolates.

Physical and biological properties of phage — Since there was no difference in the plaque size and titre of the representative isolates of both the groups of phages, XMP–7 belonging to group I i.e. lysing lac—isolates, was selected for the study of biological and physical properties which are given in Fig. 1. The highest effective

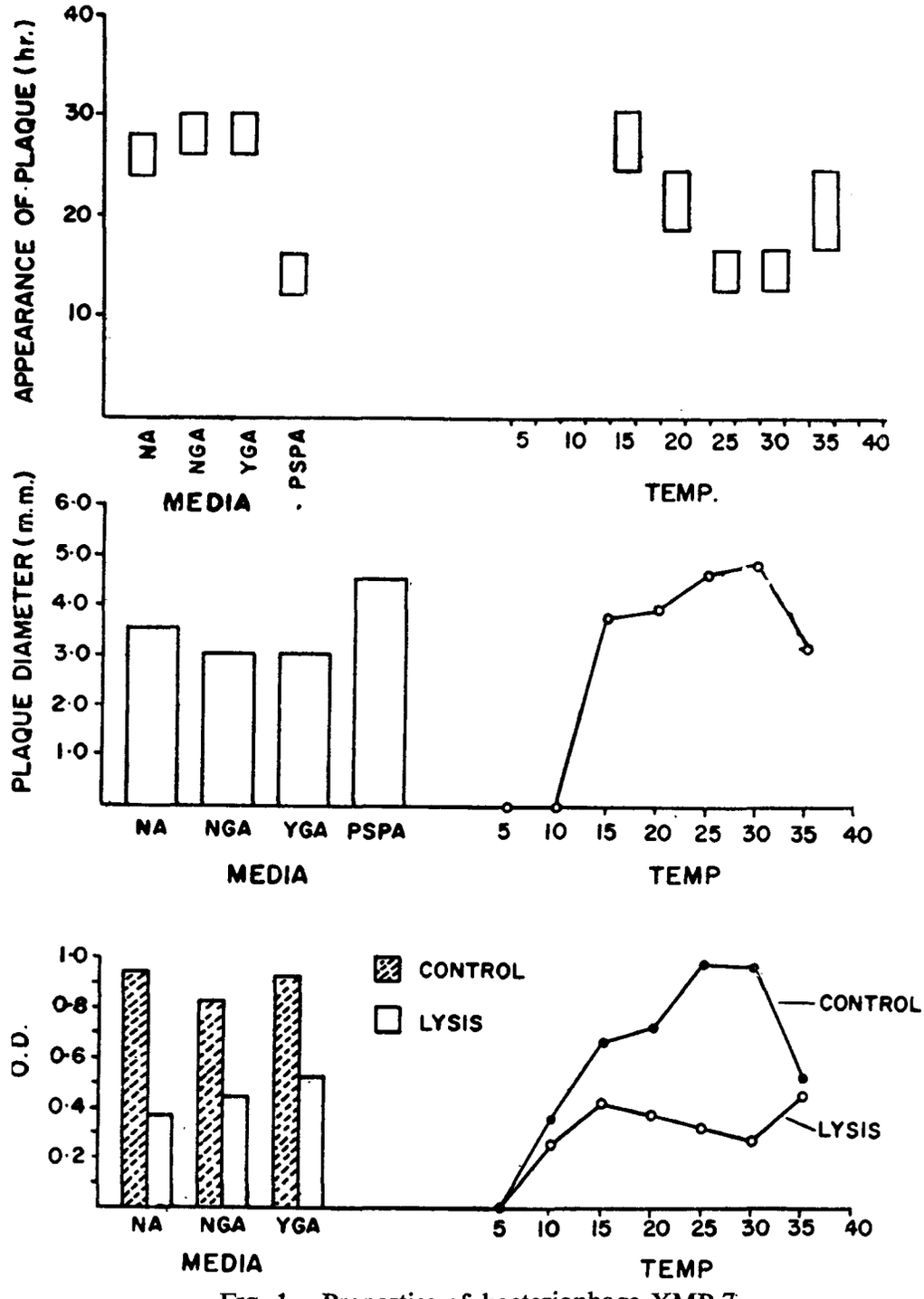

FIG. 1. Properties of bacteriophage XMP-7

dilution was determined to be 10^{-10} and the number of plaques at this dilution was 8. Thus the titre of the phage (Luria 1953) was 8×10^{10} phage particles/ml. The lysis was maximum in nutrient broth (NB), followed by nutrient glucose broth (NGB) and yeast extract glucose broth (YGB). NB was, therefore, selected for further studies. The optimum temperature for the lysis was found to be the same as the growth of the bacterium, i.e. around 30°C. The best temperature for the development and appearance of the plaques on PSPA medium was 25-30°C. The TIP (thermal inactivation point) of the phage was determined to be 75–80°C (Fig. 1).

The largest diameter of the plaques was obtained on PSPA medium (4.52 mm), followed by NA (3.55 mm). The plaques appeared on PSPA medium within 12–16 hr

whereas on the other media within 24–30 hr. The plaques were clear, circular with entire margins.

Grouping of Xanthomonas malvacearum *isolates by phage typing*—Fifty isolates of the bacterium were tested against 23 phage isolates by spot test and pour plate methods. Only 30 isolates were lysed by one group of 9 phages (which lysed lac— isolates and belonged to group I, and other 20 isolates by another group of 14 phages (which lysed lac+ and belonged to group II). It is thus observed that phages are specific to a particular biochemical group of the bacterial isolates, lysing either lac+ or lac— cultures. Further, it is concluded that on the basis of phage reaction the isolates of *X. malvacearum* could be divided into two groups only.

Discussion

In the present studies 23 phages were isolated from infected plant material, soil and water. Bacteriophages were highly specific to *X. malvacearum* and did not go to other *Xanthomonas* spp. tested (Singh *et al.* 1970). The bacteriophages were present in all the diseased *G. hirsutum* material. It could also be recovered from 2 years old material (leaf) stored under dry conditions. Massey (1931, 1935) recovered the specific phages to *X. malvacearum* from contaminated soil and water samples of blue Nile, while Roseberg and Parrack (1955) obtained phage from 2-year-old diseased cotton tissue.

The phage titre was determined to be 8×10^{10} particles/ml. Hayward (1964) observed phage titre to be in the range of 10^9 to 5×10^{10} particles/ml.

The optimum temperature for the lysis and plaque formation was observed to be between 25–30°C, and thermal inactivation at 75–80°C. Levedeva (1937) reported that thermal inactivation point lies between 56–59°C, but he exposed the phages at this temperature for 60 min.

Among the media used, the largest plaque size was obtained on PSPA medium (4.52 mm). Hayward (1964) also observed that plaque size varied from 3–5 mm on yeast extract potato agar medium within 24 hr at 25°C. Plaque size appears to vary with the media and their concentration (Matsumoto and Huzioka 1938; Okabe 1938). Matsumoto and Huzioka (1938) determined the best temperature for lytic activity to be near 28°C.

Bacteriophages could be divided into two distinct groups on the basis of their lysis to lactose utilising and nonutilising isolates. Phage group-I lysed only lac— isolates of the bacterium and were ineffective against lac+ isolates. Similarly group II phages were ineffective to lac— isolates and lysed only lac+ isolates. Similar characterisation was made by Hayward (1964).

Preliminary results in the detection of *X. malvacearum* in the seeds and other host tissues including debris with the help of phages has given encouraging results. However, the importance of using both group I and II phages must be mentioned so that either lac+ or lac— bacterial isolate could be characterised, because both these groups have been identified in the most virulent race 10 of *X. malvacearum* which attacks the maximum number of major blight resistant genes, i.e. B_2, B_7, BIN, BN.

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