TABLE III. Comparison of Zinc and EDTA in Promoting Growth of Zinc Deficient Poults.

Level of EDTA tested (mg/kg)	Zinc (mg/kg) equivalent to 100 mg/kg EDTA in diet—Exp 1	2	3	4
50	9.6	7.0		
100	7.4	9.5	5.5	8.5
200	4.7	8.5	8.8	7.7
300			7.8	
50 (with 7-10 mg/kg Zn)	17.6	27.0		
100 (with 7-10 mg/kg Zn)	16.0	15.0	19	17.5

cates that EDTA not only increases the availability of the zinc already present in the diet, but improves the availability of added zinc as well.

Summary. Various levels of EDTA and zinc were added to the poult diet containing isolated soybean protein. One hundred mg of EDTA/kg were equivalent to approximately 8 mg/kg zinc in supplementing the basal diet. When 7 or 10 mg of zinc/kg were added to the diet, 100 mg of EDTA/kg were equivalent to approximately 19 mg of zinc/kg. The data indicate that EDTA improves the effectiveness of added zinc as well as increasing the utilization of zinc already present in the basal diet. Perosis increased as the zinc level of the diet increased to approximately 30 mg/kg and decreased as zinc was added above this level.

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Lysogeny and Phage Resistance in *Pseudomonas aeruginosa.** (28386)

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Pseudomonas aeruginosa, strain Ps-7, has been shown to produce a proteolytic enzyme which contributes to the virulence of this organism in experimental infections of the cornea(1). Further studies regarding the physiology of this organism have revealed that this strain is lysogenic for at least 3 bacteriophages designated 7v, 7m and 7s. Phage 7v can be isolated from clear plaques which appear spontaneously in areas of confluent growth when Ps-7 is streaked on solid media. Phages 7m and 7s were isolated from plaques which were formed when supernatant fluids from centrifuged broth cultures of Ps-7 were mixed with a series of indicator strains of *Ps. aeruginosa* and plated by the soft agar layer method of Adams(2). Since it is well established that the presence of prophage in a lysogenic bacterium can influence many host characteristics, it was of interest to study the bacteriophages associated with strain Ps-7. Before detailed studies could be undertaken, it was important to search for non-lysogenic strains of *Ps. aeruginosa* which might serve as hosts for these phages and to compare the phages 7v and 7m with the host ranges of other *Ps. aeruginosa* bacteriophages described in the literature(3,4). Data concerning host range patterns of 12 bacteriophages and lysogenicity patterns of 95 strains of *Ps. aeruginosa* are presented here. Some of the properties of phage 7s have been reported elsewhere(5).

Materials and methods. Bacterial strains and bacteriophages. A total of 95 strains of *Ps. aeruginosa* for use in bacteriophage host range determinations and for lysogenicity assays were collected from various New Orleans hospitals. Five bacteriophages designated 12175-B1, -B2, -B3, -B4 and 12055-

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B2 were obtained from the American Type Culture Collection(6). They previously have been used for phage typing of *Ps. aeruginosa* (4). These 5 phages and phage 7v were propagated on bacterial strain Ps-7. An additional 5 phages designated A3, B3, D3, E79 and F116(3) and *Ps. aeruginosa* strains 1 and 2(7) were kindly sent to us by Dr. B. W. Holloway, Dept. of Bacteriology, University of Melbourne. The latter 5 phages were all propagated on *Pseudomonas* strain 1. Bacterial strains Ps-1 and Ps-9 are from a collection of *Ps. aeruginosa* strains isolated in this laboratory. Phage 7m was originally isolated from plaques formed on Ps-9 but was propagated on a sensitive strain designated Ps-105.

Stock phage lysates. For each phage, 25-30 tubes containing 6.5 ml melted soft agar held at 45°C were inoculated with sufficient phage and propagating bacteria to produce confluent lysis when layered over pre-poured plates containing a basal medium. The composition of the soft agar and basal medium has been reported(5). After overnight incubation at 37°C, the soft agar layers were scraped off into cold nutrient broth, homogenized 3 minutes in the presence of chloroform in a Waring blendor and left in the cold for 3 to 4 hours to permit elution of the phage from the soft agar. The homogenates were then centrifuged 45 minutes at 8,000 $\times$ *g* and the clear supernates used as stock phage preparations. Virus titers, as determined by the soft agar layer method, ranged from 1×10^{10} to 5×10^{11} pfu/ml for the 12 phages. The titers of all 12 phage stocks remained stable over a 12-month period.

Maintenance and growth of bacteria. All strains of *Ps. aeruginosa* were maintained as stab cultures in Difco stock culture agar and stored in the cold. Transfers to fresh medium were made at least once every 10 weeks. To obtain bacterial growth for bacteriophage host range determinations or for lysogenicity assays, each strain was inoculated into a liquid medium consisting of Difco nutrient broth, 0.5% yeast extract (BBL) and 1% glucose, then incubated overnight on a shaker at 34°C.

Lysogenicity assays. Ps. aeruginosa strains 1, 2, Ps-1, Ps-7 and Ps-9 were selected as indicator strains for the following reasons: strains 1 and 2 were used by Holloway as indicators to show that over 45% of approximately 200 strains of *Ps. aeruginosa* isolated in Melbourne hospitals were lysogenic(8), thus these 2 strains have a past history as useful indicators; strains Ps-1 and Ps-7 are both sensitive to phage 7v and it seemed of importance to learn if similar phages were carried by other lysogenic organisms; similarly, strain Ps-9 was included because it is sensitive to phage 7m. Bacterial strains to be assayed for lysogenicity were grown overnight and centrifuged 20 minutes at 6,000 $\times$ *g*. The supernatant fluids were stored over chloroform in screw-capped bottles. To assay for phage, 2 or 3 drops of an overnight culture of an indicator strain and 0.1 ml of culture supernatant to be tested were mixed in 3.5 ml of melted soft agar at 45°C and poured over an agar plate. After the agar layer had hardened, the plates were inverted and incubated overnight at 37°C. A given strain of *Ps. aeruginosa* was considered to be lysogenic if separated, well defined plaques were formed on one or more of the indicator strains.

Determination of phage host ranges. Bacterial lawns of each of the 95 strains of *Ps. aeruginosa* were prepared by pouring 3.5 ml melted soft agar over an agar plate after 2 or 3 drops of an overnight bacterial culture had been added. Suspensions of each of the 12 bacteriophages were prepared in nutrient broth in concentrations 100 times that required to yield confluent lysis on the propagating strain. After the bacterial lawns had dried at 37°C for 30 to 45 minutes, drops of each phage suspension were transferred by means of capillary pipettes; following an 18 hour incubation at 37°C the plates were examined for the presence of plaques. In recording the results, no attempts at quantitation were made; a given bacterial strain was considered to be either sensitive or insensitive to a particular bacteriophage. As controls, suspensions of each phage were plated on propagating bacterial strains 1 and Ps-7.

TABLE I. Host Ranges of 12 *Pseudomonas aeruginosa* Bacteriophages on Indicator Strains 1, 2, Ps-1, Ps-7 and Ps-9.

	Bacteriophages											
Source of phage	Ps-7	ATCC 12175				ATCC 12055	Ps-7	Holloway (3)				
Original designation	7v	B1	B2	B3	B4	B2	7m	A3	B3	D3	E79	F116
Assigned number	1	2	3	4	5	6	7	8	9	10	11	12
Bacterial indicator strains												
1	—	—	—	—	—	—	—	+	+	+	+	+
2	+	+	+	+	+	+	—	+	—	—	+	—
Ps-1	+	+	+	+	—	+	—	+	—	—	+	—
Ps-7	+	+	+	+	+	+	—	—	—	—	—	—
Ps-9	—	—	—	—	—	—	+	+	—	—	+	—

+ = ability to form plaques. — = no plaques formed.

Experimental results. A summary of the 12 bacteriophages used and the origin of each phage is presented in Table I. Each phage was assigned a number from 1 to 12 to facilitate recording host range patterns. Host ranges of the 12 phages tested on 5 strains of *Ps. aeruginosa* that served as indicators of lysogenicity are recorded. Phage 7v and the 5 ATCC phages formed plaques on bacterial strain Ps-7, but not on strain 1. On the other hand, all of Holloway's phages were capable of plaque formation on strain 1, but were incapable of plaque formation on strain Ps-7.

When the 95 strains of *Ps. aeruginosa* were tested for lysogenicity, it was found that all were lysogenic for a phage or phages which could form plaques on at least 1 of the 5 indicator strains. The patterns of lysogenicity obtained with respect to the 5 indicator strains are recorded in Table II. Depending upon the number of indicators on which plaques were formed, it was possible to divide the 95 bacterial strains into 5 main groups; 4 of the 5 groups could be further subdivided on the basis of the lysogenicity patterns obtained.

The results of the bacteriophage host range determinations are presented in Tables III and IV. As recorded in Table III, it was possible to divide the 12 bacteriophage strains into 3 groups on the basis of their ability to form plaques on each of the 95 bacterial strains tested. It is of interest that phage E79 which shows properties associated with virulent phages(3) exhibited the widest host range, while phage F116, which has been used to demonstrate transduction in *Ps. aeruginosa*(9), shows an extremely limited host range. The fact that no 2 of the phages tested exhibited identical host ranges is illustrated in Table IV. The phage sensitivity patterns obtained in this study would appear to be of limited value in terms of classifying the 95 strains of *Ps. aeruginosa* into distinct phage sensitivity types; from the data presented in Table IV it is possible

TABLE II. Patterns of Lysogenicity in Relation to Indicator Strains 1, 2, Ps-1, Ps-7 and Ps-9 for 95 Strains of *Pseudomonas aeruginosa.*

Carriage of a temperate phage active on indicator strain					No. of such strains isolated
1	2	Ps-1	Ps-7	Ps-9	
+	+	+	+	+	20
+	+	+	+	—	4
+	+	+	—	+	12
+	+	—	+	+	2
+	—	+	+	+	2
—	+	+	+	+	1
+	+	+	—	—	1
+	+	—	—	+	3
+	—	—	+	+	1
—	—	+	+	+	1
+	—	+	—	+	3
—	+	+	+	—	3
—	+	—	+	+	1
+	+	—	+	—	1
+	+	—	—	—	6
—	+	—	—	+	6
—	+	+	—	—	3
—	+	—	+	—	2
—	—	+	—	+	1
+	—	—	—	—	3
—	+	—	—	—	12
—	—	+	—	—	2
—	—	—	+	—	3
—	—	—	—	+	2
		Total			95

+ = Formation of plaques — = No plaques.

TABLE III. Classification of 12 *Pseudomonas aeruginosa* Bacteriophages on the Basis of Ability to Form Plaques on 95 Strains of *Pseudomonas aeruginosa*.

Group	Phage	Total No. of bacterial strains on which plaques were formed
A	E79	36
	7v	28
	12175-B1	26
	12175-B2	23
	12175-B3	25
B	12175-B4	11
	12055-B2	13
	A3	9
	B3	6
C	D3	2
	7m	2
	F116	1

to postulate 38 different groups, with the majority of groups consisting of a single bacterial strain. The largest group, consisting of 30 strains, was completely resistant to all 12 bacteriophages.

If one takes the number of indicator strains on which plaques are formed as a measure of degree of lysogenicity of a given bacterial strain, then from the data recorded in Table V, it appears that bacteria which are lysogenic for more than 1 phage are more likely to be resistant to a series of bacteriophages used for phage host range studies. It should be pointed out that a lysogenic bacterium may appear to be resistant to phage as a result of 2 different phenomena(14). First, the bacterium may be resistant in the sense that a given phage is unable to adsorb to the bacterial cell wall. On the other hand, a given bacterial type may be lysogenic for phages related to those used in the host range study. In this instance the phage may be able to adsorb to the bacterial cell wall, but is incapable of plaque formation due to immunity directed by the related prophage.

Discussion. In recent years several independent attempts have been made to classify strains of *Ps. aeruginosa* by means of sensitivity patterns obtained in response to a series of bacteriophages(4,10,11,12). Although these attempts have been made in widely scattered parts of the world, they share 2 observations in common. First, from 10% to 30% of the strains tested in any one attempt have been classified as untypable in response to a given set of bacteriophages. In the instances cited, the proportion of untypable strains has been reduced, by addition to the typing set, of phages isolated from lysogenic strains of *Ps. aeruginosa*. A second factor common to the studies reported is the observation that a set of typing phages which appears to be useful for typing strains of *Ps. aeruginosa* isolated in one geographical location, usually appears to have little utility in classifying strains of this organism isolated in a different part of the world. The occurrence of lysogeny in *Ps. aeruginosa* is well documented(3) and is implied, although not thoroughly investigated, by workers attempting to develop sets of typing phages. The data obtained in this study tend to support an earlier observation by Holloway(8) that patterns of lysogenicity obtained in relation to a set of indicator strains can serve as a useful method for grouping strains of *Ps. aeruginosa*. Since 90% of our *Pseudomonas* strains were found to by lysogenic when using indicator strains isolated in Australia

TABLE IV. Patterns of Phage Sensitivity in Relation to 12 *Pseudomonas aeruginosa* Bacteriophages for 95 Strains of Lysogenic *Pseudomonas aeruginosa*.

Sensitivity to bacteriophages*	No. of such strains isolated	Sensitivity to bacteriophages*	No. of such bacterial strains isolated
1,2,3,4,5,6,8,11	2	1,5,6	1
1,2,3,4,5,8,10,11	1	1,3,4	2
1,2,3,4,5,6,11	1	2,9,11	1
1,2,3,4,6,8,11	1	2,3,5	2
1,4,5,8,9,11	1	1,3,4	1
1,2,5,8,9,11	1	2,4,11	1
1,3,4,6,9,11	1	2,3	1
2,3,4,5,8,11	1	2,5	8
1,2,3,4,5,6	1	2,11	1
8,9,10,11,12	1	1,11	2
1,3,4,6,11	1	1,4	1
1,3,7,8,11	1	1,6	2
1,2,3,4,6	1	3,4	1
1,3,4,6	2	11	10
5,8,9,11	1	2	5
1,3,4,11	1	8	1
2,4,8,11	1	4	2
1,2,4,6	1	1	1
1,3,6,9	1	Resistant	30
2,8,11	1	Total	95

* Each of the 12 bacteriophages was assigned a number 1-12 shown in Table I.

TABLE V. Relation Between Lysogenicity as Evidenced by Plaque Formation on 5 Indicator Strains and Phage Resistance in 30 Strains of *Pseudomonas aeruginosa.*

Lysogenic for bacteriophage which formed plaques on	No. of such strains isolated	No. resistant to all 12 phages	% resistant to all 12 phages
All 5 indicator strains	20	14	70
4/5 of indicator strains	21	5	23
3/5 " " "	14	6	42
2/5 " " "	18	3	16
1/5 " " "	22	2	9
Totals	95	30	—

it seems that results obtained by this method are not greatly influenced by the geographical location where the indicator strains were originally isolated. The data presented here also suggest that the majority of strains of *Ps. aeruginosa* are lysogenic for more than one bacteriophage. Such a situation can lead to confusion in interpretation of phage sensitivity patterns when these strains are used as propagating bacteria for preparation of stock phage lysates(13).

Summary. When 95 strains of *Ps. aeruginosa* were assayed for lysogenicity on a series of indicator strains, all bacteria were found to be lysogenic. From the patterns of lysogenicity obtained with respect to the indicators we conclude that the majority of the 95 strains tested were lysogenic for more than one bacteriophage. When the patterns of lysogenicity obtained in this study were compared with patterns of phage sensitivity with respect to 12 bacteriophages, it appeared that the lysogenicity patterns showed more utility with respect to grouping strains of this organism. Approximately 30% of the strains tested were found to be untypable with respect to the bacteriophages used, whereas all strains could be typed on the basis of lysogenicity patterns. Finally, the resistance of a given strain of *Ps. aeruginosa* to a series of bacteriophages appeared to be related to the degree of lysogenicity exhibited on a series of indicator bacteria.

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