Phage-Host Specificity Tests using *Levinea* Phages and Isolates of *Levinea* spp. and *Citrobacter freundii*

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Phage-host specificity tests were accomplished utilizing six serologically unrelated phages with 177 isolates of the genus *Levinea* and 44 *Citrobacter freundii* isolates. A total of 84% of the *Levineae* were infected by at least one of the phages, and 21 phage-host patterns were obtained. Only 9% of the *C. freundii* isolates were infected. One phage, DM-21, only infected *L. malonatica* whereas the other phages showed no species specificity. Most of the infectivity patterns were specific for either *L. amaltonatica* or *L. malonatica*, but some patterns showed no host specificity. A correlation was found between dulcitol fermentation and infectivity with DM-21. There was no evidence of a relationship between infectivity and source of isolation. The recommendation that the proposed genus *Levinea* be accepted rather than the inclusion of these organisms within the genus *Citrobacter* is discussed.

With the dichotomy which exists as to the classification of the *Levinea* organisms, a unique opportunity arose to utilize the *Levinea* phages isolated by Markel and Eklund (7, 7a) as a taxonomic tool to demonstrate whether a relatedness existed between the genera *Levinea* and *Citrobacter*. Major biochemical and serological differences between the two genera have been described and discussed previously (7, 12). It is apparent that these organisms are being isolated more frequently in the United States (1, 6, 9, 10) and abroad (5, 8, 13) in clinical laboratories, and the need for an agreement on nomenclature is obvious.

The purpose of this study was to test both *Levinea* and *Citrobacter* isolates for infectivity by the *Levinea* phages. The possible use of the phage infectivity patterns for species identification of *Levinea* was examined. Correlation between phage infectivity and the hosts' biochemical reactions was also investigated. (This investigation was presented in part at the March 1974 meeting of the Texas Branch of the American Society for Microbiology, Houston, Texas.)

Bacterial cultures. From July 1968 to January 1974, the Texas State Health Department (TSHD) at Austin, Texas, accumulated 285 enteric isolates which were qualified to be classified in the genus *Levinea* on the basis of key biochemical reactions. From these, 173 were randomly selected for use in phage susceptibil-

ity tests. There were 57 cultures of *Levinea amaltonatica*, 108 cultures of *Levinea malonatica*, and 8 cultures of *Levinea* intermediates. A reference strain of each *L. amaltonatica* and *L. malonatica* used in the isolation of the phages DM-11, DM-21, and DM-31, as well as *L. amaltonatica* (ATCC 25405) and *L. malonatica* (ATCC 25408) utilized in the isolation of DM-41, DM-51, and DM-61, was included (7, 7a). A total of 177 *Levinea* organisms were tested. Screening criteria for the genus *Levinea* and *Citrobacter freundii* are given in Table 1. Species differentiation of *Levinea* is based on the possible combination of three biochemical reactions which are listed in Table 2 along with the corresponding reactions for *Citrobacter*. Also tested were 27 isolates of H₂S-positive *C. freundii* and 16 H₂S-negative *C. freundii* variants which were randomly selected from TSHD isolates and an H₂S-positive *C. freundii* strain (ATCC 8090). A total of 44 *Citrobacter* isolates were tested.

Phages. The six *Levinea* phages previously reported (7, 7a) were used in the phage-host susceptibility tests.

Methods. The methods used in the preparation of indicator organisms and host specificity tests have been previously described (7).

Phage-host specificity tests. The percentage of the *Levinea* and *Citrobacter* organisms infected by at least one *Levinea* phage is presented in Table 3. None of the six phages were infective for any of the H₂S-positive *Citrobacter* isolates and a very low percentage

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of infection was obtained for the H₂S-negative *Citrobacter* variants. A high percentage of *Levinea* cultures was infected.

The host specificity tests are segregated into species infected by the individual phages in Table 4. Five of the six phages lacked species specificity; however, DM-21 appeared to be infective only for *L. malonatica*.

Phage infectivity patterns. Phage infectivity patterns of the 177 *Levinea* isolates are presented in Table 5. Patterns 1 to 10 were specific for *L. malonatica* whereas patterns 11 to 14 were specific for *L. amalonatica*. Six patterns showed no species specificity. One pattern included those organisms which were not infected by any of the six phages.

Correlation of phage susceptibility with the hosts' biochemical characteristics and sources of isolation. Results indicated that DM-21 was infective for 29 of 110 *L. malonatica* isolates. Of these 29 organisms, 20 (69%) were dulcitol positive. It was also noted that all 59 isolates of *L. amalonatica* were dulcitol negative and were not infected by DM-21. No one infectivity pattern was specific for the dulcitol fermentation; however, 45% of the infected organisms had a pattern consistent with number 6 in Table 5. There was no apparent correlation between source of the isolates and phage infectivity patterns.

Phages are usually host specific in that their infectivity has been restricted to closely related species of a single genus. However, this specificity is not always observed in the genera included

in the family *Enterobacteriaceae*. Some phages have been isolated that can infect more than one host genus. These observations have been postulated to be due to the fact that this family contains separate genera that are closely related but differ in certain key biochemical reactions (2).

The opportunity to utilize the *Levinea* phages as taxonomic tools to yield evidence as to the classification of levineae has been obvious. If these phages showed a high infectivity for the typical *C. freundii*, then the contention of Ewing and Davis that the organisms are to be classified as *Citrobacter* would be apparent and justified (4). As the results indicated, such was not the case. There was no infectivity for the typical H₂S-positive *C. freundii*. The small number of atypical H₂S-negative *C. freundii* organisms which were infected was not significant as compared to the high percentage of the *Levinea* organisms which were infected (Table 3).

Phage infectivity patterns indicated that the phages could be useful in identification of levineae since a number of patterns were specific for only one species. These patterns were reproducible when random samples were selected for retesting.

Correlation was observed between dulcitol fermentation and DM-21 infectivity. No correlation between phage infectivity and source of isolation was apparent.

TABLE 1. Biochemical screening criteria for *Levinea* with corresponding reactions for *C. freundii*

Biochemical reactions	<i>Levinea</i>	<i>C. freundii</i>
Triple sugar iron agar	Acid butt, H ₂ S -	Acid butt, H ₂ S +
Indole	+	-
Methyl red	+	+
Simmons' citrate	+	+
Phenylalanine deaminase	-	-
Lysine decarboxylase	-	-
Ornithine decarboxylase	+	-

TABLE 3. Number of organisms infected by at least one *Levinea* phage

Organism	No. of isolates	No. infected	%
<i>Levinea amalonatica</i>	59	43	73
<i>Levinea malonatica</i>	110	99	90
Group III	7	5	71
Group IV	1	1	100
Total	177	148	84
<i>Citrobacter freundii</i>			
H ₂ S-positive	28	0	0
H ₂ S-negative	16	4	25
Total	44	4	9

TABLE 2. Biochemical tests for species differentiation of *Levinea*

Test	Group I	Group II	Group III	Group IV	<i>C. freundii</i>
Adonitol	+	-	-	+	-
Malonate	+	-	+	-	-
KCN	-	+	Vary	Vary	+
Designation	<i>L. amalonatica</i>	<i>L. malonatica</i>			<i>C. freundii</i>

TABLE 4. Number of isolates infected by each *Levinea* phage

	DM-11	DM-21	DM-31	DM-41	DM-51	DM-61
<i>Levinea malonatica</i>	1	30	81	5	65	68
<i>Levinea amalonatica</i>	5	0	24	2	3	38
Group III	0	0	4	0	5	4
Group IV	0	0	1	0	0	1
<i>Citrobacter freundii</i>						
H ₂ S positive	0	0	0	0	0	0
H ₂ S negative	0	0	1	0	0	4

TABLE 5. Phage-host infectivity patterns

Pattern	DM-11	DM-21	DM-31	DM-41	DM-51	DM-61	Total %	Organisms infected ^a
1.	+	+	+	+	+	+	0.6	<i>L.m.</i>
2.	-	+	+	+	+	+	0.6	<i>L.m.</i>
3.	-	+	+	+	-	+	0.6	<i>L.m.</i>
4.	-	+	+	-	+	-	2.8	<i>L.m.</i>
5.	-	+	+	-	-	-	1.1	<i>L.m.</i>
6.	-	+	+	-	-	+	4.0	<i>L.m.</i>
7.	-	+	+	-	-	-	6.3	<i>L.m.</i>
8.	-	+	-	-	-	-	0.6	<i>L.m.</i>
9.	-	-	+	+	+	+	1.1	<i>L.m.</i>
10.	-	-	-	-	+	+	2.8	<i>L.m.</i>
11.	+	-	+	-	-	+	1.1	<i>L.a.</i>
12.	+	-	-	-	-	+	1.7	<i>L.a.</i>
13.	-	-	+	+	-	-	0.6	<i>L.a.</i>
14.	-	-	-	+	-	+	0.6	<i>L.a.</i>
15.	-	-	+	-	-	-	3.5	<i>L.a.</i> , <i>L.m.</i>
16.	-	-	+	-	-	-	4.0	<i>L.a.</i> , <i>L.m.</i>
17.	-	-	-	-	-	+	9.6	<i>L.a.</i> , <i>L.m.</i>
18.	-	-	+	-	+	+	23.7	<i>L.a.</i> , <i>L.m.</i> , Grp III
19.	-	-	+	-	-	+	13.0	<i>L.a.</i> , <i>L.m.</i> , Grp IV
20.	-	-	-	-	+	-	6.8	<i>L.m.</i> , Grp III
21.	-	-	-	-	-	-	15.8	<i>L.a.</i> , <i>L.m.</i> , Grp III

^a *L.a.*, *L. amalonatica*; *L.m.*, *L. malonatica*.

In conclusion, the authors recommend that the generic designation *Levinea*, as proposed by Young et al. (12) be accepted rather than placing the organisms within the genus *Citrobacter* for the following reasons: (i) biochemical tests have clearly demonstrated a separate genus (12); (ii) phage-host specificity tests demonstrated no relatedness between *Levinea* and *Citrobacter*; (iii) Young et al. (12) reported no serological relatedness between *Levinea* and *Citrobacter*, yet all members of the tribe *Salmonellae* have been reported to be serologically related (3); (iv) the addition of *C. diversus* as a second accepted species within the *Citrobacter* genus would necessitate revision of the genus as well as the tribe *Salmonellae* (4); (v) *C. diversum*, originally described by Werkman and Gillen (11), differs from *C. diversus* as described by Ewing and Davis (4) in that it was described as H₂S positive, gave an indefinite methyl red test, and was nonmotile. Negative

H₂S production and a positive methyl red reaction are distinctive biochemical characteristics of the genus *Levinea* and most isolates are motile.

REPRINT REQUESTS

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