Comparison of Several Methods for Preserving Bacteriophages

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Received for publication April 24, 1962

ABSTRACT

CLARK, WILLIAM A. (American Type Culture Collection, Washington, D. C.). Comparison of several methods for preserving bacteriophages. Appl. Microbiol. **10**:466–471. 1962—A wide variety of bacteriophages were processed and stored under different conditions to compare methods for long-term preservation. Specimens were stored for 2 years at room temperature (24 to 28 C) and at 4 C as broth lysates in 50% glycerol, dried, and freeze-dried. Titers determined after processing indicated that, of the broth, glycerol, and freeze-dry methods, freeze-drying was most damaging to the phages tested, glycerol less damaging, and the broth method least damaging. After 2 years, titers of broth lysates were generally higher than those of glycerol or freeze-dried preparations. Dried preparations generally did not prove satisfactory. Preparations stored at 4 C showed better titers than those kept at room temperature. All titers declined with time regardless of the conditions of preservation.

In 1954 the American Type Culture Collection (ATCC) began to accumulate a collection of documented bacteriophage strains for long-term preservation and distribution. In this connection, it became essential to determine the best practical means for preserving phage strains unchanged for long periods.

Different methods for preserving viruses, including phages, have been used. They include refrigeration (Adams, 1950), storage with glycerol or other preservatives (Rhoads, 1929; Rivers and Ward, 1935), drying (Prouty, 1953), and freeze-drying (Harris, 1954).

The purpose of the present study was to compare the effectiveness of those methods of preserving phage.

MATERIALS AND METHODS

Strains of phage used in these studies, their hosts, media and temperature for propagation are listed in Table 1.

Phage strains were propagated in broth according to standard methods (Adams, 1950). All phage lysates were centrifuged (unless complete lysis occurred), then filtered through Selas #03 porcelain filters.

Phage filtrates were kept refrigerated (4 C) until a convenient time for processing, then assayed by a modification of Asheshov and Heagy's (1951) "loop" method (a 0.025-ml ultra-micropipette was substituted for Asheshov's "big loop" to reduce error in the method), processed for long-term preservation according to methods described below, reassayed, and stored in duplicate both at room temperature (uncontrolled, 24 to 28 C) and in the refrigerator (4 to 6 C). Phages were reassayed quantitatively (except for those preserved on filter paper, which were assayed only for viability) after storage for 1 and 2 years.

Phage hosts generally were preserved by the method of freeze-drying routinely practised at the ATCC and subsequent storage in the refrigerator at 4 C. *Vibrio comma* 12174 failed to survive freeze-drying by this method, hence it was preserved in "NIH semi-solid medium" (*see* Weiss, 1957) under mineral oil at room temperature. (This strain appears to survive best under these conditions.)

Processing phages for long-term storage. Phages were processed for long-term storage (i) by dispensing the broth lysate into 4-ml screw-capped vials (no. 60910; Kimball Glass Co., Toledo, Ohio) having vinylite plastic-lined caps for refrigerated storage at 4 C; (ii) by adding sufficient sterile glycerol (cp) to the broth lysate to make a 50% (v/v) "solution" and dispensing as above; (iii) by saturating filter-paper circles (Whatman no. 1, average ash 0.00020 g) with phage lysate, and then keeping them at 2 to 5 C over anhydrous calcium sulfate in an evacuated vacuum desiccator at 2 to 5 C until visibly dry; and (iv) by freeze-drying the lysate mixed with an equal volume of sterile, double-strength, skim milk (Difco) as routinely practised at the ATCC (Weiss, 1957).

No special cleaning procedures were followed with the glass vials used, except for a thorough rinsing with demineralized water.

Storage conditions. Vials in which lysates and lysate-glycerol mixtures were stored were carefully capped and tightened to avoid evaporation. All preparations were stored in duplicate. When a defective seal permitted perceptible evaporation the preparation in question was discarded.

Filter-paper circles containing dried phage were placed in screw-capped vials, which were tightly capped after the drying process was complete, and stored in a desiccator.

Reconstitution and assay of phage after storage. Both lysate alone and lysate with glycerol were assayed by removing samples from the storage vials and titering by the method of Asheshov and Heagy (1951).

Lyophilized phage was assayed by first rehydrating the dried pellet in exactly 1 ml of appropriate broth con-

taining approximately 10^8 host cells per ml and then titering by Asheshov and Heagy's (1951) method.

Phage dried on filter-paper circles was assayed only for viability as follows. Duplicate circles were placed on appropriate agar slants seeded with the host, wetted with a loopful of suitable broth, and then incubated. Viability was indicated by lysis of the host in the immediate area of each paper square.

Results

The data for titrations of the phages stored for a 2-year period are shown in Table 2. Titers shown for phages treated with glycerol and by freeze-drying have been adjusted to account for dilution during processing and reconstitution.

Effects of prestorage treatment. No tests were made to determine the effect of lowering the temperature of the phage strains from room temperature to 4 C. It is assumed that no loss in titer occurred.

All phage strains tested survived treatment with 50% glycerol except one, *Bacillus brevis* 10027-B. Survivors showed varying degrees of sensitivity to glycerol (Table 3). Exposure to 50% glycerol of the phages tested generally appears to have had less harmful effects than did treatment by freeze-drying.

All phage strains tested survived freeze-drying except for *B. brevis* 10027-B. Freeze-drying generally had a more drastic effect on the phages tested than did exposure to 50% glycerol (Table 3).

Of 14 phage strains tested immediately after drying, 12 withstood such treatment.

Effect of storage temperature on long term survival. The effects of temperature on stored phages are shown in Table 4. Phages generally survived better at 4 C than at room temperature, regardless of the manner in which they were processed prior to storage. Exceptions were phage 11634-B for *Serratia marcescens*, which survived equally well in broth in either temperature range, and phage 12016-B for the thermophilic *Bacillus* sp., which survived in glycerol equally well at either temperature and survived drying better at room temperature. Phage 12060-B_1 for *B. polymyxa* showed better survival when freeze-dried and kept at room temperature than when kept at 4 C.

Effects of storage conditions only. Table 4 shows generally the effects of storage on phage viability, not counting damage due to prestorage treatment.

In 11 instances where the data permit comparison of survival under different storage conditions at room temperature, ten phages showed best recovery when lyophilized, and one showed best survival in glycerol.

In 15 instances where the data permit comparison of effects of the various storage conditions at 4 C, eight phages showed best recovery from freeze-dried preparations, four from broth and three from 50% glycerol.

Of 11 phage strains viable after drying, 2 survived 2 years of storage at 26 C, and 6 at 4 C.

Combined effects of prestorage treatment and storage con-

TABLE 1. *Bacteriophages used, with conditions for propagating in studies of preservation of phages*

Phage strain	Host	Propagation of phage	
		Temp	Medium
		C	
10027-B[a]	*Bacillus brevis* 10027[a]	37	Nutrient broth (Difco)
11478-B	*B. megaterium* 11478	25	*B. megaterium* broth[b]
12060-B_1	*B. polymyxa* 12060	25	Nutrient Broth (Difco)
12060-B_4	*B. polymyxa* 12060	25	Nutrient Broth (Difco)
12139-B	*B. subtilis* 12139	25	Papain horsemeat broth[c]
12016-B	*Bacillus* sp. (thermophilic) 12016	55	Trypticase Broth (supplemented)[d]
8677-B	*Escherichia coli* 8677	37	Nutrient Broth (Difco)
11303-B_1–B_7	*E. coli* 11303	37	Nutrient Broth (Difco)[e]
11727-B	*Mycobacterium smegmatis* 11727	37	Glycerol medium[f]
11953-B	*Pasteurella pestis* 11953	37	CTA Medium (BBL)
12055-B_1	*Pseudomonas aeruginosa* 12055	37	Nutrient Broth (Difco)
11954-B	*Rhizobium leguminosarum* 11954	25	Waksman's no. 79 medium[g]
11634-B	*Serratia marcescens* 11634	37	Nutrient Broth (Difco)
11987-B	*Staphylococcus aureus* 11987	37	Trypticase Soy Broth (BBL)
11988-B	*S. aureus* 11988	37	Trypticase Soy Broth (BBL)
12174-B_1	*Vibrio comma* 12174	37	Papain horsemeat broth[c]

[a] ATCC accession number (American Type Culture Collection, 1958).

[b] Yeast Extract (Difco), 2.5 g; Trypticase (BBL), 1 g; agar, 18 g; distilled water, 1 liter.

[c] Asheshov (1941).

[d] Trypticase Broth (Baltimore Biological Laboratory, Inc., Baltimore, Md.; BBL), 2 g; $FeCl_3$, 0.0007 g; $MgSO_4$, 0.0015 g; distilled water, 100 ml.

[e] Supplemented with 0.5% NaCl added to medium.

[f] Weiss (1957).

[g] Mannitol, 10 g; K_2HPO_4, 0.5 g; $MgSO_4$, 0.2 g; NaCl, 0.1 g; $CaCO_3$, 3.0 g; Yeast Extract (BBL), 0.5 g; distilled water, 1 liter; agar, 15 g (pH 7.2).

TABLE 2. *Titers of some bacteriophages stored under different conditions*

Bacteriophage strain	Host	Year	Titer of phage[a]							
			In broth		In 50% glycerol		Lyophilized		Dried	
			26 C	4 C	26 C	4 C	26 C	4 C	26 C	4 C
10027-B	*Bacillus brevis* 10027	1955	1.3×10^{7b}	1.3×10^{7}	0[c]	0	0[c]	0	0	0
		1957	0	+	0	0	0	0	0	0
11478-B	*B. megaterium* 11478	1955	5.2×10^{7}	5.2×10^{7}	4.8×10^{7}	4.8×10^{7}	2.4×10^{7}	2.4×10^{7}	—	—
		1957	+	—	0	3.4×10^{6}	7.7×10^{7}	1.2×10^{7}	0	—
12060-B_1	*B. polymyxa* 12060	1955	2.0×10^{9}	2.0×10^{9}	5.2×10^{8}	5.2×10^{8}	2.3×10^{7}	2.3×10^{7}	+	+
		1957	0	5.7×10^{6}	0	1.7×10^{7}	1.6×10^{7}	4.8×10^{6}	0	+
12060-B_4	*B. polymyxa* 12060	1955	9.8×10^{7}	9.8×10^{7}	3.6×10^{7}	3.6×10^{7}	1.8×10^{6}	1.8×10^{6}	+	+
		1957	0	1.7×10^{6}	0	1.9×10^{7}	—	4.8×10^{6}	0	+
12139-B	*B. subtilis* 12139	1955	1.8×10^{9}	1.8×10^{9}	9.4×10^{8}	9.4×10^{8}	4.0×10^{8}	4.0×10^{8}	+	+
		1957	+	1.4×10^{8}	0	1.8×10^{8}	—	3.8×10^{7}	0	0
12016-B	*Bacillus* sp. (thermophilic) 12016	1955	5.3×10^{7}	5.3×10^{7}	5.6×10^{7}	5.6×10^{7}	2.6×10^{7}	2.6×10^{7}	+	+
		1957	+	9.6×10^{4}	3.2×10^{5}	7.6×10^{5}	3.2×10^{6}	2.4×10^{7}	+	0
8677-B	*Escherichia coli* 8677	1955	8.8×10^{9}	8.8×10^{9}	7.2×10^{9}	7.2×10^{9}	1.3×10^{6}	1.3×10^{6}	+	+
		1956	—	9.5×10^{9}	5.1×10^{6}	5.6×10^{9}	1.1×10^{6}	7.7×10^{6}	—	—
		1957	5.1×10^{8}	1.1×10^{10}	6.4×10^{4}	1.2×10^{10}	2.0×10^{8}	4.5×10^{8}	0	+
11303-B_1	*E. coli* 11303	1955	8.4×10^{10}	8.4×10^{10}	7.6×10^{9}	7.6×10^{9}	2.7×10^{9}	2.7×10^{9}	+	+
		1956	2.5×10^{9}	8.8×10^{9}	2.1×10^{8}	4.2×10^{9}	6.4×10^{6}	1.1×10^{7}	—	—
		1957	1.5×10^{8}	2.0×10^{9}	2.9×10^{6}	4.7×10^{8}	6.8×10^{8}	1.9×10^{9}	—	—
11303-B_2	*E. coli* 11303	1955	9.2×10^{9}	9.2×10^{9}	2.6×10^{7}	2.6×10^{7}	4.2×10^{7}	4.2×10^{7}	+	+
		1956	2.5×10^{9}	2.2×10^{10}	9.6×10^{7}	7.1×10^{7}	1.1×10^{6}	—	—	—
		1957	4.6×10^{7}	3.9×10^{10}	—	2.4×10^{8}	7.9×10^{5}	6.9×10^{6}	0	+
11303-B_3	*E. coli* 11303	1955	8.5×10^{7}	8.5×10^{7}	1.6×10^{7}	1.6×10^{7}	1.1×10^{7}	1.1×10^{7}	+	+
		1956	9.6×10^{6}	—	0	—	3.6×10^{5}	—	—	—
		1957	3.3×10^{8}	1.4×10^{8}	5.0×10^{2}	1.8×10^{5}	2.6×10^{6}	3.5×10^{6}	0	+
11303-B_4	*E. coli* 11303	1955	1.2×10^{9}	1.2×10^{9}	2.2×10^{7}	2.2×10^{7}	1.5×10^{8}	1.5×10^{8}	—	—
		1956	—	—	—	—	3.2×10^{5}	—	—	—
		1957	6.4×10^{7}	2.8×10^{9}	0	2.8×10^{7}	—	—	0	0
11303-B_5	*E. coli* 11303	1955	4.2×10^{10}	4.2×10^{10}	4.2×10^{10}	4.2×10^{10}	6.6×10^{9}	6.6×10^{9}	—	—
		1956	1.2×10^{10}	1.4×10^{10}	7.6×10^{8}	1.8×10^{10}	3.0×10^{6}	8.0×10^{6}	—	—
		1957	2.9×10^{9}	2.9×10^{10}	2.4×10^{8}	1.3×10^{10}	8.3×10^{8}	3.5×10^{9}	0	0
11303-B_6	*E. coli* 11303	1955	2.3×10^{10}	2.3×10^{10}	1.1×10^{9}	1.1×10^{9}	2.3×10^{9}	2.3×10^{9}	—	—
		1956	3.7×10^{9}	1.3×10^{10}	3.2×10^{8}	4.2×10^{8}	4.7×10^{5}	8.9×10^{5}	—	—
		1957	7.3×10^{8}	1.1×10^{10}	7.8×10^{7}	7.6×10^{8}	9.6×10^{6}	1.0×10^{8}	0	0
11303-B_7	*E. coli* 11303	1955	4.8×10^{9}	4.8×10^{9}	6.4×10^{9}	6.4×10^{9}	1.2×10^{9}	1.2×10^{9}	+	+
		1956	1.1×10^{6}	—	7.6×10^{5}	2.8×10^{9}	1.2×10^{6}	4.2×10^{6}	—	—
		1957	+	2.6×10^{9}	0	2.8×10^{8}	1.8×10^{7}	2.0×10^{8}	+	+
11727	*Mycobacterium smegmatis* 11727	1956	5.2×10^{7}	5.2×10^{7}	5.4×10^{7}	5.4×10^{7}	1.1×10^{6}	1.1×10^{6}	—	—
		1957	+	4.1×10^{8}	+	2.9×10^{7}	+	1.0×10^{8}	0	0
11953-B	*Pasteurella pestis* 11953	1955	1.8×10^{6}	1.8×10^{6}	5.0×10^{5}	5.0×10^{5}	0	0	—	—
		1957	0	1.3×10^{6}	+	+	+	+	—	0
12055-B_1	*Pseudomonas aeruginosa* 12055	1956	1.6×10^{8}	1.6×10^{8}	9.6×10^{7}	9.6×10^{7}	7.4×10^{5}	7.4×10^{5}	—	—
		1957	+	1.6×10^{8}	+	4.2×10^{7}	+	+	0	0
11954-B	*Rhizobium leguminosarum* 11954	1955	1.5×10^{7}	1.5×10^{7}	2.4×10^{7}	2.4×10^{7}	5.9×10^{6}	5.9×10^{6}	+	+
		1957	0	1.3×10^{7}	0	1.3×10^{6}	1.9×10^{6}	+	0	0
11634-B	*Serratia marcescens* 11634	1955	4.3×10^{8}	4.3×10^{8}	1.6×10^{8}	1.6×10^{8}	0	0	0	0
		1957	5.6×10^{7}	4.3×10^{7}	0	0	3.8×10^{4}	0	0	0
11987-B	*Staphylococcus aureus* 11987	1955	3.6×10^{8}	3.6×10^{8}	5.8×10^{8}	5.8×10^{8}	9.2×10^{7}	9.2×10^{7}	+	+
		1957	0	4.9×10^{7}	0	2.4×10^{7}	1.5×10^{7}	3.1×10^{7}	0	0
11988-B	*S. aureus* 11988	1955	1.9×10^{8}	1.9×10^{8}	1.9×10^{8}	1.9×10^{8}	2.3×10^{5}	2.3×10^{5}	+	+
		1957	0	4.7×10^{7}	0	1.3×10^{7}	0	+	0	0
12174-B	*Vibrio comma* 12174	1956	9.6×10^{7}	9.6×10^{7}	4.6×10^{7}	4.6×10^{7}	3.9×10^{5}	3.9×10^{5}	—	—
		1957	0	1.4×10^{7}	0	6.0×10^{5}	—	2.6×10^{6}	0	0

[a] Symbols: + = filtrate viable but titer too low to count by method used; 0 = nonviable; — = titer not determined.
[b] Titer before processing for long-term storage.
[c] Titer after processing for long-term storage.

TABLE 3. *Effects of prestorage treatment on phages*

Phage	Per cent survival after treatment with 50% glycerol	Per cent survival after freeze-drying	Per cent survival after drying[a]
Bacillus brevis 10027-B	0	0	0
B. megaterium 11478-B	92	46	—
B. polymyxa 12060-B_1	26	1	+
B. polymyxa 12060-B_4	37	2	+
B. subtilis 12139-B	52	22	+
Bacillus sp. (thermophilic) 12016-B	>100	49	+
Escherichia coli 8677-B	82	<1	+
E. coli 11303-B_1	9	3	+
E. coli 11303-B_2	<1	<1	+
E. coli 11303-B_3	18	13	+
E. coli 11303-B_4	2	12	−
E. coli 11303-B_5	100	16	−
E. coli 11303-B_6	5	10	−
E. coli 11303-B_7	>100	25	+
Mycobacterium smegmatis 11727-B	>100	2	−
Pasteurella pestis 11953-B	28	0	−
Pseudomonas aeruginosa 12055-B_1	60	<1	−
Rhizobium leguminosarum 11954-B	>100	33	+
Serratia marcescens 11634-B	37	0	0
Staphylococcus aureus 11987-B	>100	25	+
S. aureus 11988-B	100	<1	+
Vibrio comma 12174-B	48	<1	−

[a] Symbols: + = viable; 0 = nonviable; —= no data.

ditions at 4 C. Table 5 attempts to assess the over-all effects of prestorage treatment and storage at 4 C on the various phages.

Of 20 phages, 13 showed highest recoveries from broth, 5 showed highest recoveries from glycerol, and only 2 showed highest recoveries from freeze-dried specimens.

All phages tested could be recovered from broth. In two cases, no recovery was obtained from either glycerol or from lyophilized specimens.

Only 6 of 20 phages survived drying over a 2-year period.

Discussion

Experiments to evaluate methods for preservation of living materials are complicated by the fact that damage, deterioration, or death may be caused by a number of factors during prestorage treatment, storage itself, or retrieval from storage of the preserved material. In the present studies, an attempt was made to assay the materials tested before treatment, immediately after treatment, 1 year after treatment, and 2 years after treatment. Under these conditions, it is difficult to determine where one effect stops and another begins. Also, these experiments were not designed to differentiate between effects of retrieval, i.e., thawing or rehydration on the one hand and those of prestorage or storage treatment on the other. Therefore, the results obtained with each phage must be viewed generally as over-all effects produced by a composite treatment.

TABLE 4. *Effects on phages of storage conditions only*

Phage	Per cent survival							
	In broth		In glycerol		Freeze-dried		Dried	
	26 C	4 C	26 C	4 C	26 C	4 C	26 C	4 C
Bacillus brevis 10027-B	0[a]	+	0	0	0	0	—	—
B. megaterium 11478-B	<1	—	0	7	>100	50	—	—
B. polymyxa 12060-B_1	0	<1	0	3	70	21	0	+
B. polymyxa 12060-B_4	0	2	0	53	—	>100	0	+
B. subtilis 12139-B	<1	7	0	19	—	10	0	0
Bacillus sp. (thermophilic) 12016-B	<1	<1	<1	1	12	92	+	0
Escherichia coli 8677-B	6	>100	<1	>100	>100	>100	0	+
E. coli 11303-B_1	<1	2	<1	6	25	70	—	—
E. coli 11303-B_2	<1	>100	—	>100	2	16	0	+
E. coli 11303-B_3	<1	>100	<1	1	24	32	0	+
E. coli 11303-B_4	5	>100	0	<100	—	—	—	—
E. coli 11303-B_5	7	69	<1	31	13	53	—	—
E. coli 11303-B_6	3	48	7	70	<1	4	—	—
E. coli 11303-B_7	<1	54	0	4	2	17	+	+
Mycobacterium smegmatis 11727-B	<1	>100	+	54	+	>100	—	—
Pasteurella pestis 11953-B	0	72	+	+	+	+	—	—
Pseudomonas aeruginosa 12055-B_1	<1	100	+	44	+	+	—	—
Rhizobium leguminosarum 11954-B	0	87	0	5	32	+	0	0
Serratia marcescens 11634-B	13	10	0	0	—	—	—	—
Staphylococcus aureus 11987-B	0	14	0	4	16	34	0	0
S. aureus 11988-B	0	25	0	7	0	+	0	0
Vibrio comma 12174-B	0	15	0	1	—	>100	—	—

[a] Symbols: + = viable, no quantitative data obtained; 0 = nonviable; — = no data.

In the present studies, viability only was tested because of limitations on staff, time, and facilities. In the ideal preservation experiments, characterization tests should be included with assays of viability to determine genetic stability of the preserved materials.

A modified method of Asheshov and Heagy (1951) was used for titering phage preparations. Despite some loss in accuracy over the standard dilution methods, this method provides a quick and relatively simple titration, useful for titering a large number of samples when time and facilities are limited.

Screw-capped vials were selected for keeping broth and glycerinated phage specimens in these experiments for convenience in titering the same preparations repeatedly. However, they have been found by us to be unsatisfactory for long-term preservation of phage specimens because of the possibility of evaporation due to occasional imperfect sealing when a cap is not properly tightened or a vinyl liner is rendered useless through autoclaving. A number of sealed glass ampules for each strain preserved would provide improved conditions for long-term preservation.

Several general observations should be made about the data obtained. It will be noticed that the effectiveness of a given method of preservation seems to hold generally for most phages tested, although, as is true with most groups of organisms in a collection, there are always individual species or even strains which do not lend themselves to a given method for preservation.

No single group of phage seems particularly sensitive to any given treatment.

In some cases, the titer of a given sample rose markedly during storage, rather than falling as one might expect it to do. The same phenomenon was noted in the work of Zierdt (1959) with staphylococcal phages. Whether this type of occurrence is an artifact or not we cannot say.

Zierdt (1959) obtained good stability of staphylococcal phages by lyophilizing and subsequently storing them at −20 C. Lyophilization is a very convenient method for preserving phage. However, during the method of freeze-drying described here, the titers generally fell considerably. The discrepancy in these results would suggest the value of a program for studying, improving, and standardizing freeze-drying procedures.

Zierdt's (1959) and our own experience with temperature effects on preserved living materials are in accord with the theoretical expectation that the lower the temperature of storage the longer a material can be preserved in the living state.

Irregardless of the method used to preserve phage, the general over-all decline of the preparations with time should be noted. Better methods must be sought to preserve phage over extended periods of time.

TABLE 5. *Combined effects of prestorage treatment and storage conditions (4 C) on phages*

Phage	Per cent survival			
	In broth	In glycerol	Freeze-dried	Dried
Bacillus brevis 10027-B	+[a]	0	0	0
B. megaterium 11478-B	—	7	23	—
B. polymyxa 12060-B_1	<1	1	<1	+
B. polymyxa 12060-B_4	2	19	5	+
B. subtilis 12139-B	8	10	2	0
Bacillus sp. (thermophilic) 12016-B	<1	1	45	0 (+ at 26 C)
Escherichia coli 8677-B	>100	>100	5	+
E. coli 11303-B_1	2	1	2	—
E. coli 11303-B_2	>100	3	<1	+
E. coli 11303-B_3	>100	<1	4	+
E. coli 11303-B_4	>100	2	—	0
E. coli 11303-B_5	69	31	8	0
E. coli 11303-B_6	48	3	<1	0
E. coli 11303-B_7	54	6	4	+
Mycobacterium smegmatis 11727-B	>100	56	>100	0
Pasteurella pestis 11953-B	72	+	+	0
Pseudomonas aeruginosa 12055-B_1	100	26	+	0
Rhizobium leguminosarum 11954-B	87	9	+	0
Serratia marcescens 11634-B	10	0	0(<1 at 26 C)	0
Staphylococcus aureus 11987-B	14	7	9	0
S. aureus 11988-B	25	7	+	0
Vibrio comma 12174-B	15	1	3	0

[a] Symbols: + = viable, no quantitative data obtained; 0 = nonviable; — = no data.

ACKNOWLEDGMENTS

Use of the facilities of the National Canners Association for some of the research reported here is gratefully acknowledged.

This investigation was supported in part by a research grant (G17185) from the National Science Foundation, and in part by a research grant (E-1534) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

LITERATURE CITED

ADAMS, M. H. 1950. Methods of study of bacterial viruses. Meth-Medical Research **2**:1–73.

AMERICAN TYPE CULTURE COLLECTION. 1958. Catalog of cultures, 6th Ed.

ASHESHOV, I. N. 1941. Papain digest media and standardization of media in general. Can. J. Public Health **32**:468–471.

ASHESHOV, I. N., AND F. C. HEAGY. 1951. A "loop" method for counting viable bacteria or bacteriophage. Can. J. Med. Sci. **29**:1–4.

HARRIS, R. J. C. 1954. The preservation of viruses, p. 201–214. *In* R. J. Harris [ed.], Biological applications of freezing and drying. Academic Press, Inc., New York.

PROUTY, C. C. 1953. Storage of the bacteriophage of lactic acid streptococci in the desiccated state with observations on longevity. Appl. Microbiol. **1**:250–251.

RHOADS, C. P. 1929. Survival of the virus of poliomyelitis for 8 years in glycerol. J. Exptl. Med. **49**:701–704.

RIVERS, T. M., AND S. M. WARD. 1935. Jennerian prophylaxis by means of intradermal injections of culture vaccine virus. J. Exptl. Med. **62**:549–560.

WEISS, F. A. 1957. Maintenance and preservation of cultures, p. 99–119. *In* H. J. Conn [ed.], Manual of microbiological methods. McGraw-Hill Book Co., Inc., New York.

ZIERDT, C. H. 1959. Preservation of staphylococcal bacteriophage by means of lyophilization. Am. J. Clin. Pathol. **31**:326–331.