Viability of Dried Bacterial Cultures

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With a Note on the Immediate Death-Rate

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SUMMARY: Cultures were dried in a vacuum over phosphorus pentoxide. At varying intervals up to 14 years tests were made on 2724 strains (representing over forty genera) and 88 % of cultures were found to be viable. *Staphylococcus*, *Sarcina* and *Micrococcus* were the genera most resistant to drying; *Vibrio* and *Neisseria* were among the least resistant.

Many methods have been described for the preservation of bacteria by drying (Hammer, 1911; Rogers, 1914, 1949; Swift, 1921, 1937; Flosdorf & Mudd, 1938; Rayner, 1943; Greaves, 1944; Stamp, 1947; Proom & Hemmons, 1949); these differ in the desiccating agent, menstruum for the bacteria, and in the complexity of the apparatus and manipulation needed. All the modern methods give satisfactory results; simplicity and efficiency are the only reasons for reporting the method to be described. The method is not original, but I cannot find any reference to it in the literature; Prof. A. Sordelli described it to Dr R. St John-Brooks and it has been in use in the National Collection of Type Cultures (N.C.T.C.) since 1934. Tubes were opened as cultures were required for distribution; there was no planned experiment, but over 15 years a mass of information has accumulated which is summarized in this paper.

METHODS

Drying. Cultures were usually grown on a solid medium for 24 hr.; cooked meat medium (Robertson's medium) was sometimes used for the anaerobes. Organisms were not dried until there was good growth; with actinomycetes and mycobacteria this sometimes took a week or more.

Rimless tubes were specially made in two sizes (Poulton, Selfe and Lee Ltd.); the smaller (8 × 60 mm.) was a neat fit in the larger (10 × 150 mm.). The smaller tubes (Fig. 1*a*) were plugged and sterilized, and until required for use were kept dry in an incubator. Identification data were written in indian ink on the small tube (white ink was unsatisfactory as it was affected by the P_2O_5). A small amount of P_2O_5 was put in the large tube; to do this without fouling the sides a glass rod and funnel with a stem 130 mm. long were used (Fig. 1*b*).

A loopful of horse serum was deposited on the inner wall of the small sterilized tube and a loopful of growth was emulsified in it. The small tube was inserted into the wider tube containing P_2O_5 and the tight fit of the cotton-wool plug prevented the smaller tube from making contact with the P_2O_5 (Fig. 1*c*). The outer tube was then constricted above the level of the wool plug

(Fig. 1*d*), and when cool was attached to a Hyvac pump. A good vacuum was obtained in 2–3 min. and the tube was sealed at the constriction (Fig. 1*e*); the vacuum was examined with a high frequency tester (W. Edwards and Co. (London) Ltd.). Cultures were stored in the dark at room temperature.

Recovery. The outer tube was opened and the inner tube removed; about 1 ml. of broth or other suitable liquid medium was pipetted into the small tube, and

Fig. 1. Stages in the preparation of dried cultures. *a*, small sterile tube with identification data; *b*, funnel, and glass rod used for putting P_2O_5 at the bottom of the large tube; *c*, small tube in larger tube, the bottom of the small tube is not in contact with the P_2O_5 ; *d*, large tube constricted above the plug of the smaller tube; *e*, sealing off the outer tube while attached to the vacuum pump; *f*, the finished product.

incubated at the optimum temperature of the organism. When turbidity developed, usually within 24–48 hr., a subculture was made on solid medium. Incubation was continued for 10–14 days (21–28 days for actinomyces and mycobacteria) before discarding tubes that failed to show growth.

Material dried. Almost every bacterium in the N.C.T.C. was dried in duplicate between 1984 and 1989; afterwards two desiccates were made of each new strain (with the exception of *Leptospira* spp.) added to the Collection.

To keep up the stock, a new dried culture was made from the growth of a tube that had been opened. If the first tube failed to grow the second tube

was opened and, if that grew, two new desiccates were made. Generally, the first tube opened was picked at random; it might have been dried for less than a month or for many years. In 1948-9 some selection was made, and, to increase the number of cultures tested after longer intervals, the older tube was chosen.

Identification of cultures and growth. Until recently the depositor's determination was always accepted. Sometimes dried cultures were made from the original culture sent to the Collection. After recovery from the dried state the identity of the culture was confirmed by inspection of the growth and by stained smears. Because of lack of time and the necessary facilities, biochemical and fermentation tests were seldom employed.

RESULTS

Just under a quarter of the tests were made on cultures that had been dried for less than a year (Table 1). Up to 11 years the survival rates varied between 79 and 88 % of tubes opened, but after longer intervals there was a sharp decrease. A possible explanation of the poor results from cultures dried in 1934 is that serum was not used when the method was first employed.

Table 1. *Viability of bacteria at different intervals after drying*

Interval	No. tested	Contaminated	Viable	Percentage viable
Less than 1 month	92	3	80	87.0
1 month	1604	34	1413	88.1
1 yr.	1004	30	854	85.1
2 yr.	674	21	572	84.9
3 yr.	409	12	344	84.1
4 yr.	291	7	287	81.4
5 yr.	295	10	258	87.5
6 yr.	257	4	217	84.4
7 yr.	293	4	233	79.5
8 yr.	357	10	282	79.0
9 yr.	448	7	376	83.9
10 yr.	491	8	414	84.3
11 yr.	428	14	346	80.8
12 yr.	162	9	111	68.5
13 yr.	154	11	103	66.9
14 yr.	169	27	78	46.1
	7128	211	5918	

Gross contamination occurred in 211 (3 %) of tubes opened, and these are included among the non-viable; in a number (not recorded) of tubes lesser degrees of contamination made it possible to recover the organism originally dried. The greatest number of contaminated tubes was found in species of *Vibrio* (52 contaminated). In other groups contamination was associated with the necessity for enrichment of the media used for growth; *Haemophilus* (32), *Neisseria* (18), *Streptococcus* (18), *Mycobacterium* (12), and *Pseudomonas* (12).

The genera tested, and their survival after different intervals, are shown in Table 2 (for species see 'List of Species', 1948). In compiling this table an attempt

Table 2. *Survival of different genera after drying*

Genus	No. of strains tested	Period (years) between drying and test					Percentage of cultures viable
		< 1	1-4	5-9	10-14	< 1-14 (Total)	
		No. of cultures viable/no. tested					
<i>Actinomyces</i> (anaerobic)	29	9/11	19/37	7/9	3/4	38/61	62
<i>Actinomyces</i> (aerobic)	20	—	13/15	7/11	2/2	22/28	79
<i>Nocardia</i>	18	4/4	3/3	13/15	4/8	24/30	80
<i>Streptomyces</i>	34	13/13	20/21	16/19	2/2	51/55	93
<i>Acetobacter</i>	34	20/20	38/42	18/22	8/9	84/93	90
<i>Pseudomonas</i>	107	34/36	76/102	46/62	66/109	222/309	72
<i>Vibrio</i>	134	56/74	53/101	32/92	11/132	152/399	38
<i>Xanthomonas</i>	36	11/14	30/39	9/9	10/14	60/76	79
<i>Mycoplana</i>	1	—	—	0/2	—	0/2	—
<i>Spirillum</i>	4	1/1	1/1	0/1	1/1	3/4	—
<i>Micrococcus</i>	72	51/51	21/21	29/29	26/26	127/127	100
<i>Sarcina</i>	11	7/7	2/2	4/4	9/9	22/22	100
<i>Staphylococcus</i>	108	54/54	132/133	47/48	30/30	263/265	99
<i>Neisseria</i>	88	62/69	66/118	38/72	22/61	188/320	59
<i>Lactobacillus</i>	35	15/17	17/22	23/29	9/12	64/80	80
<i>Leuconostoc</i>	13	8/10	20/24	5/5	7/7	40/46	87
<i>Streptococcus</i>	316	398/445	567/610	171/178	129/136	1265/1369	92
<i>Bacillus</i>	240	55/69	87/95	169/183	122/128	433/475	91
<i>Clostridium</i>	24	6/11	12/17	8/13	1/3	27/44	61
<i>Flavobacterium</i>	7	7/7	12/14	3/3	0/1	22/25	88
<i>Chromobacterium</i>	26	7/10	22/27	9/16	8/14	46/67	69
<i>Bacterium</i> (coli-aerogenes)	113	69/69	75/78	70/71	55/59	269/277	97
<i>Bacterium</i> (<i>Erwinia</i>)	28	10/10	14/18	15/16	16/23	55/67	82
<i>Bacterium</i> (non-fermenting)	11	5/5	5/7	6/7	6/6	22/25	88
<i>Bacterium</i> (paracolon)	42	10/10	8/8	28/29	18/18	64/65	98
<i>Bacterium</i> (uncertain status)	67	37/46	31/44	32/39	23/30	123/159	77
<i>Salmonella</i>	246	29/29	70/73	144/146	69/76	312/324	96
<i>Shigella</i>	130	46/46	88/95	73/78	57/62	264/281	94
<i>Proteus</i>	104	52/54	48/48	39/44	72/77	211/223	95
<i>Mycobacterium</i>	106	52/55	49/61	50/71	51/61	202/248	81
<i>Corynebacterium</i>	154	71/72	98/104	97/102	52/59	318/337	94
<i>Erysipelothrix</i>	27	2/2	5/6	13/13	17/17	37/38	97
<i>Zopfius</i>	7	1/2	1/1	7/7	6/6	15/16	94
<i>Actinobacillus</i>	10	11/13	18/24	3/5	7/11	39/53	74
<i>Brucella</i>	58	40/48	33/46	26/31	55/72	154/197	78
<i>Haemophilus</i>	110	191/237	159/199	33/56	15/26	398/518	77
<i>Pasteurella</i>	58	31/34	59/62	35/42	33/37	158/175	90
<i>Pfeifferella</i>	10	—	7/8	5/6	5/7	17/21	81
<i>Fusiformis</i>	17	3/5	9/28	2/7	1/9	15/49	31
<i>Azotobacter</i>	15	2/5	—	5/10	6/13	13/28	46
<i>Leptothrix</i>	3	—	—	0/5	—	0/5	—
<i>Microspira</i>	1	0/1	0/1	1/3	—	1/5	—
<i>Photobacterium</i>	9	4/16	1/3	3/5	0/2	8/26	31
<i>Propionibacterium</i>	11	5/5	5/5	16/17	2/2	28/29	97
<i>Pleuro-pneumonia</i>	6	2/6	10/12	5/6	5/6	22/30	73
<i>Vibriothrix</i>	2	—	—	2/2	—	2/2	—
<i>Rhizobium</i>	26	1/1	2/2	2/9	9/15	14/27	52
<i>Sporocytophaga</i>	1	1/1	—	—	1/1	2/2	—
<i>Thiobacillus</i>	1	0/1	1/1	0/1	1/1	2/4	—

has been made to place species in the appropriate genus; for example, strains received as *Salmonella morganii* are shown under *Proteus*, but because complete characterization was not undertaken in the Collection, some strains may have been seriously misplaced. With most genera a high proportion of tubes were viable up to 10–12 years and little comment is needed. *Pseudomonas trifoliorum*, *Vibrio cholerae*, *Brucella tularensis* and *Mycobacterium murium* were species that did not survive for long periods. Four strains of *Chromobacterium violaceum* soon died out but another strain lived for 5 years.

In general, the *Bacillus* group dried well but *B. repens* was an exception; of two strains tested, one (N.C.T.C. 7126) was not recovered from either tube, and the other (N.C.T.C. 4820) only from two of four tubes. Most *Clostridium* species survive well in meat cultures, consequently few were dried. Four strains of *Cl. acetoniigenum* were dried but only three of eleven tubes were alive; sand cultures (Thaysen, 1924) of this organism, received from Dr A. C. Thaysen, grew after storage for at least 10 years.

The results obtained with *Rhizobium* spp. must be interpreted with caution; when these strains were received bodies resembling spores were seen in stained films and doubt was felt about the correctness of the determination. Unfortunately, before drying the cultures films were not stained specifically for spores. When tested, most tubes gave a growth of *Bacillus circulans*, some a mixture of *Rhizobium* and *Bacillus circulans*.

Fungi and yeasts were dried by the same method and the results have been reported (Rhodes, 1950).

Effect of storage temperature

An experiment was made to see if dried cultures could be sent by post to the tropics. Six tubes of *Staphylococcus aureus* and six tubes of *Vibrio cholerae* were dried and kept at 37°. Two years later all tubes of the *staphylococcus* were viable; all those of the *vibrio* were dead. These results are not included in Tables 1 or 2.

DISCUSSION

Survival rates in the tables refer to the number of tubes that gave growth on test, and not to the proportion of organisms that were viable. Experiments made recently by P. J. Fisher show that the method (slightly modified in that a measured volume was dried) may kill from 20 to 99 % of bacteria in suspension during the drying period. With such a high immediate death-rate it is essential that the suspension to be dried should be heavy and consist mainly of living organisms. It follows, therefore, that the optimal time to dry the culture is at the end of the logarithmic phase. Disregard for this principle, as in the chromobacteria where growth was continued until pigment had developed, and the use of the depositor's original cultures, may account for the poor results obtained with species such as *Chromobacterium violaceum* and *Mycobacterium murium*.

In general, the results agree with those of Stamp (1947) and of Proom & Hemmons (1949) who found *Vibrio cholerae* and the pathogenic species of

Neisseria viable only for short periods, while streptococci and staphylococci survived the longest time tested.

It is to the interest and encouragement of Dr R. St John-Brooks, who started drying cultures by the method outlined in this paper, that I owe my thanks.

A Note on the Immediate Death-Rate

By P. J. FISHER

Without modification, Sordelli's method cannot be controlled by viable counts of the organism before and after drying. To do this the volume of suspension must be increased, and this will inevitably decrease the rate of drying. Experience has shown that an increase in the drying time is accompanied by an increase in the killing effect of the process. It follows, therefore, that the death-rates reported in this note are probably greater than those obtained in the routine use of Sordelli's method as described by Miss Rhodes.

Table 3. *Viable counts before and after drying*

Organism	Strain N.C.T.C. no.	Viable count/ml.		Death- rate (%)
		Before	After	
<i>Bacterium coli</i>	86	38×10^8	24×10^8	37
<i>Streptococcus pneumoniae</i>	2428	134×10^8	110×10^8	18
<i>Vibrio cholerae</i>	7270	80×10^8	83×10^8	99
<i>Neisseria gonorrhoeae</i>	6822	40×10^4	32×10^8	92

A measured volume of suspension was dried in vacuum over P_2O_5 ; using the roll-tube method (Wilson, 1922) viable counts were made of the suspension to be dried and of the dried product after reconstitution to the original volume. Table 3 shows the results of four typical experiments, two made with species known to survive for a long time, and two with species that do not dry well. The immediate death-rate, calculated from the counts made before and 24 hr. after drying, varied from 18 % with *Streptococcus pneumoniae* to 99 % with a freshly isolated strain of *Vibrio cholerae*.

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