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Viability of *Actinomycetales* Stored in Soil[1]

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About 1,800 *Actinomycetales* stored in soil for up to 20 years were checked for viability. About one-half were viable.

Since the early 1950s, we have used soil culture, agar slant culture, and lyophilization concomitantly for preservation of all strains of aerobic *Actinomycetales*, mostly streptomycetes and streptoverticillia. The large number of strains accumulated over the years has forced abandonment of routine transfer on agar slants and, more recently, the use of soil cultures. The decision to abandon preservation by soil culture prompted examination of these materials to determine how many had survived storage at room temperature over periods up to about 20 years. The information obtained is presented herein, along with some miscellaneous observations relating to this collection.

Three different kinds of soil cultures had been prepared. An initial lot had been made by adding suspensions of spores to sterilized loamy soil. These preparations, stored in a refrigerator under humid conditions, became contaminated with molds and, for the most part, had to be destroyed. A second lot of soil cultures was prepared by adding broth cultures to sterilized prairie black loam soil and allowing them to dry at room temperature (2) with subsequent storage at room temperature. In 1959, a third lot of cultures was prepared in a silica sand-$CaCO_3$ amended soil (screened, air-dried prairie black loam soil, 10,000 g; white silica sand, 7,500 g; and $CaCO_3$, 25 g; mixed by hand and screened).

Because of the possibility of mite infestation, the cotton stoppers of one lot of tubes were treated with a miticide (0.1% $HgCl_2$ in 95% ethanol containing identification dye). Unfortunately, this lot was additionally sealed with rubber stoppers over the cotton plugs to facilitate numbering of tubes for rapid retrieval from large storage racks. Many of these cultures died, possibly because of the exclusion of air or permeation of the soil with ethanol, or both. New soils had to be prepared for each of these after only short periods of storage (up to 1 year). With the passage of time, much dust accumulated on the cotton stoppers. Despite this, few cultures were found to be contaminated (Table 1).

Viabilities were determined by adding six loopsful (0.5 mm inside diameter of loop, dampened) of soil from each culture to a tube of tryptone-glucose-liver extract-yeast extract broth (1), incubating with shaking for 72 h at 28 to 30 C, and pipetting approximately 0.2 ml of this inoculum onto a yeast extract agar slant

TABLE 1. *Viability of soil cultures of Actinomycetales over a 20-year period*

Year of preparation	Age at time of test (years)	No. of cultures tested	No. of cultures viable	No. of cultures contaminated
1951-1955[a]	17-21	433	276	26
1956	16	166	100	10
1957	15	161	97	6
1958	14	360	217	13
1959[b]	13	19	12	0
1960	12	126	68	5
1961	11	160	62	13
1962	10	81	48	2
1963	9	65	34	2
1964	8	21	8	2
1965	7	105	34	2
1966	6	8	2	0
1967	5	37	9	4
1968	4	16	1	2
1969	3	22	12	1
1970	2	33	7	2
1971	1	0	0	0
1972	1	2	1	0
Totals		1,815	988	90

[a] These tubes were not dated but had been prepared in the period from 1951 through 1955.

[b] In 1959 the loamy soil-silica sand-$CaCO_3$ mixture was instituted for preparation of soil cultures.

[1] This study was presented, in part, at the 73rd annual meeting of the American Society for Microbiology, Miami Beach, Fla., 6-11 May 1973.

and approximately 0.5 ml onto inorganic salts-starch agar (2) in a petri dish. Inoculated slants and dishes were held for 10 days at 28 to 30 C and checked for growth as a measure of viability.

About one-half of the tested soil cultures contained viable *Actinomycetales* (Table 1). Age of storage seemed to have little effect on the number of strains viable. The results suggest some other reason for death of the cultures, e.g., the method of preparation, the drying out process, or exposure to fluctuating room temperature.

Our experiences with these soil cultures and with maintenance on agar slants is an argument in favor of lyophilization as a means of preservation. Lyophilized preparations have shown much higher percentage of survival and less risk of contamination of the cultures based on the experiences of the several curators of the Agricultural Research Service Culture Collection over a 25- to 30-year period.

B. P. was an Agency for International Development scholarship trainee, Department of Health, Bangkok, Thailand.

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