

Microbial Culture Preservation With Silica Gel

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Cessation of cellular metabolism is a prerequisite for the preservation and long-term storage of a microbial culture in a state essentially free from the accumulation of morphological and physiological variants (Reusser, 1963). The method of lyophilization (Heckley, 1961) is the most universally used procedure for stock culture preservation.

Hunt, Gourevitch & Lein (1958) and Perkins (1962) described methods for preserving microbial cultures by dehydration with anhydrous silica gel. The method of Hunt *et al.*, where silica gel and culture are held apart, has been used to preserve fungi (Lange & Boyd, 1968) and a range of bacterial species (Hunt *et al.* 1958; Norris, 1963). The technique reported by Perkins, in which a suspension of the micro-organism in skim-milk is added directly to the anhydrous silica gel, has been applied to stocks of the fungi *Neurospora crassa*, *Ustilago maydis*, strains of yeast (Perkins, 1962), cyst-forming slime moulds (Reinhardt, 1966) and *Claviceps paspali* (Mizrahi & Miller, 1968). This technique is inexpensive, rapid, extremely simple to use and, as reported in this communication, can also be applied to micro-organisms other than fungi.

METHODS

The experimental procedure used was that described by Perkins (1962) for fungal species. Each of the microbial species tested was grown in the appropriate medium specified in Table 1. For *Azotobacter vinelandii*, the medium contained (g./l. double-distilled water): sucrose, 10; K₂HPO₄, 2; MgSO₄·7H₂O, 0·4; Na-citrate, 0·2; NaCl, 0·1; CaCl₂, 0·05; FeSO₄·7H₂O, 0·01; Na₂MoO₄·2H₂O, 0·002; sufficient HCl to adjust the pH to 7·6; NH₄Cl, 0·1 was added for stock reactivation only. With the exceptions of *Aspergillus nidulans* and *Pseudomonas denitrificans*, the organisms were cultured in aerated liquid media and collected by centrifuging at 5000 *g* for 5 to 10 min. in sterile plastic centrifuge tubes sealed with screw caps. The cells were resuspended in reconstituted skim-milk (15%, w/v; Bonlac obtained from Trufood, Glenormiston, Victoria), previously autoclaved at 121° for 10 min. *A. nidulans* conidia and *P. denitrificans* cells were suspended in milk (7·5%, w/v) added to cultures growing on agar slopes. It may be that the presence of the skim-milk is a requirement for the successful preservation of bacterial strains by this method (see Hunt *et al.* 1958; Reinhardt, 1966).

Cotton-wool stoppered 13 × 100 mm. Pyrex glass tubes were half filled with silica gel (grade as specified by Perkins, 1962) and dry-sterilized at 175° for 1·5 to 2 hr. Pre-cooled suspension (0·5 ml.) was added dropwise to each chilled tube of anhydrous

silica gel. These were held at 0° for a further 10 to 15 min., then kept for one week in a desiccator containing activated silica gel, prior to a viability test. Finally, each tube was sealed with Parafilm and stored over self-indicating silica gel in a sealed jar at 2° to 4°.

Reactivation of a dehydrated culture was accomplished by incubating several granules of the silica gel stock in the appropriate liquid medium, the remaining dehydrated material being resealed and stored as described. Thus, each tube of dehydrated culture may be used repeatedly. The medium used to reactivate each culture was the same as that in which it had been grown initially except in the case of *Azotobacter vinelandii* (above). No estimates were made of relative viabilities.

The metabolic characteristics of the bacteria were tested as described by the following authors: Brownell & Nicholas (1967) for nitrogen fixation by intact organisms; Radcliffe & Nicholas (1968) and Lam & Nicholas (1969) for nitrate and nitrite dissimilation; Adams (1950) for bacteriophage sensitivity.

Table 1. *Culture conditions and periods of survival of micro-organisms successfully preserved on anhydrous silica gel*

Species	Strain	Period of survival at last testing (weeks)	Culture medium and temperature
<i>Saccharomyces cerevisiae</i>	DELFT 171	46	YEPD medium (Manney, 1964); 30°
<i>Aspergillus nidulans</i>	bi-1	37	Minimal medium (Cove, 1966) + 1 g./l., KNO ₃ ; 30°
<i>Anabaena cylindrica</i>	—	107	Nitrate medium (Brownell & Nicholas, 1967); 25–28°
<i>Azotobacter vinelandii</i>	ATCC 13705	103	See text; 30°
<i>Escherichia coli</i>	B	46	Hershey's Nutrient Broth (Chase & Doermann, 1958) + 0.5 g./l. extra glucose; 37°
	BB	45	
	HS	45	
	B-185	45	
	K ₁₂ ^λ	66	
	830	45	
<i>Pseudomonas denitrificans</i>	ATCC 13867	111	Nutrient agar (Radcliffe & Nicholas, 1968); 37°
<i>Micrococcus denitrificans</i>	NCIB 8944	61	Nutrient agar (Radcliffe & Nicholas, 1968); 37°
<i>Thiobacillus concretivorus</i>	NCIB 9514	13	Thio-oxidans medium (Vishniac & Santer, 1957) with trace elements diluted ten times; 30°

RESULTS AND DISCUSSION

Table 1 lists the microbial species which survived dehydration satisfactorily on anhydrous silica gel, and includes details of the culture conditions. To date, we have been unable to recover viable cells from dehydrated cultures of *Thiobacillus thiparus*, *Chlamydomonas eugametos* and *Euglena gracilis*.

All reactivated cultures had the expected macroscopic and microscopic appearance

and, when tested, the bacterial strains retained characteristic metabolic properties. Thus, *Azotobacter vinelandii* intact cells fixed nitrogen gas, *Pseudomonas denitrificans* and *Micrococcus denitrificans* dissimilated inorganic nitrogen compounds, *Thiobacillus concretivorus* lowered the pH of thiosulphate medium to less than 2, and the several strains of *Escherichia coli* (excepting the T₄-resistant strain, 830) were susceptible to infection by bacteriophage T₄.

This technique of stock culture preservation was particularly useful in simplifying basic microbiological manipulations in an essentially biochemical laboratory. It is recommended for its extreme simplicity and apparent reliability.

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