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The Preservation of Bacteria and Fungi on anhydrous Silica Gel: an Assessment of Survival over Four years

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The preservation method of Perkins (1962) using suspensions in skim-milk was used to preserve 33 bacteria and 22 fungi on anhydrous silica gel. During storage at room temperature, 64% of the bacteria and 77% of the fungi survived 1 year or more. Storage at 4° often increased the survival period *c.* 2- to 3-fold: 73% of the bacteria and all 12 of the fungi tested at 4° survived > 1 year. At the last testing, 60% of the bacteria and 36% of the fungi were still viable after storage at 4° for periods between 3 and 4 years. The Gram positive bacilli tended to survive the silica gel preservation process better than most Gram negative bacilli. Some factors influencing survival after preservation on silica gel are discussed; the results support the use of a closed storage tube.

PERKINS (1962) described a preservation method whereby fungal spores or mycelium suspended in skim-milk were adsorbed on anhydrous silica gel particles. The original culture could be reconstituted by shaking out a few granules on an appropriate culture medium. One anhydrous stock tube could be sampled repeatedly over several years to yield identical inocula free from mutation and the method was simple, convenient and did not need expensive equipment.

Perkins applied his method to 700 strains of *Neurospora crassa* and subsequently this technique has been used successfully with > 1000 strains of *Neurospora* spp. (Barratt, Johnson & Ogata, 1965; Sargent & Woodward, 1969; Benveniste & Munkres, 1970; De Serres & Smith, 1970; Schroeder, 1970). Other fungi preserved successfully were *Aspergillus nidulans* (Barrett *et al.*, 1965), *Asp. parasiticus* (Mayne, Bennett & Tallant, 1971), *Rhodotorula* sp. (Reinhardt, 1966), *Claviceps paspali* (Mizrahi & Miller, 1968), *Ustilago maydis* (Puhalla, 1968), *Endothia parasitica* (Puhalla & Anagnostakis, 1971), *Saccharomyces cerevisiae* (Grivell & Jackson, 1969), *Candida* spp. (Parina, Patrikeev & Lysenko, 1972), *Fusarium oxysporum* and *Verticillium* spp. (Rogers, 1972).

The blue green alga *Anabaena cylindrica* (Grivell & Jackson, 1969) and the cellular slime moulds *Acrasis rosea* and *Dictyostelium discoideum* (Reinhardt, 1966; Watts & Ashworth, 1970) were preserved successfully. Reinhardt also recorded the successful but short-term preservation of the bacterium *Escherichia coli* included as a food source. Better survival of *E. coli* was obtained later by Grivell & Jackson (1969) and Brown (1971). Other bacteria which survived this preservation technique were *Azotobacter vinelandii, Pseudomonas denitrificans, Micrococcus denitrificans, Thiobacillus concretivorus* (Grivell & Jackson, 1969). The technique was used to preserve plant viruses (lettuce necrotic yellows, tomato spotted wilt, cucumber mosaic, cauliflower mosaic) by Grivell, Grivell, Jackson & Nicholas (1971).

In the majority of the cases cited, the successful storage period has been 1–3 years but for 4 organisms the period was less, i.e. 6 months (3 months in one instance). For *N. crassa* (Perkins, 1962) and *Asp. parasiticus* (Mayne *et al.*, 1971) 5–7 years is reported. The Fungal Genetics Stock Center, California maintains conidial strains of *Neurospora* spp. and of *Asp. nidulans* on anhydrous silica gel.

The silica gel preservation method is reported to be unsuccessful for *Chlamydomonas eugametos*, *Euglena gracilis* and *Thiobacillus thioparus* (Grivell & Jackson, 1969), streptomyces (Hopwood & Ferguson, 1969; Kutzner, 1972) and flexibacteria (Sanfilippo & Lewin, 1970).

The present report, covering nearly 4 years' storage, assesses the application of the silica gel preservation method to a range of bacteria and fungi for which information was not available and assesses storage at room temperature.

Materials and Methods

Preservation on silica gel

The procedure used was modified from the methods described by Perkins (1962) and Grivell & Jackson (1969). Each organism, obtained from the collections listed (Tables 1, 2), was grown on a slope of the appropriate medium (Tables 1, 2) until good confluent growth was obtained: 1–3 days for bacteria, 3–5 days for fungi. The cells were suspended in sterile distilled water, previously cooled to 4°, added to the culture slope. An equal volume of chilled reconstituted skim-milk (15% w/v; Marvel, Cadbury Schweppes Foods, Bournville, Birmingham; sterilized at 121° for 10 min) was added to produce the final cell suspension. The silica gel (6–18 mesh, Fisons Scientific Apparatus Ltd, Loughborough, Leicestershire) was sterilized (180° for 1·5–2·0 h) in Pyrex glass test tubes, 12 × 75 mm, with cotton-wool plugs. Each chilled tube, *c.* half full of silica gel granules, received 0·5 ml of pre-cooled cell suspension which was distributed slowly and evenly on the granules. These tubes were stored in close-fitting boxes or desiccators containing self-indicating silica gel as desiccant at either room temperature or 4°. For some cultures, silica gel preparations were made in screw-capped containers (¼ oz and 1 oz) which were sealed during storage.

Viability assessment

The viability of each cell suspension preserved on silica gel granules was determined by placing several granules on the appropriate agar medium and incubating at the optimum temperature. The correct character of the resultant culture was verified macroscopically and/or microscopically. Viability was assessed during the initial 1–3 weeks and subsequently at irregular intervals during storage. From the results of successive tests, a reduction in viable numbers was inferred if there was an increase in either the incubation period before macroscopic growth became visible or in the percentage of silica gel granules failing to yield macroscopic growth.

Results

The 33 different bacterial cultures which survived dehydration on silica gel are listed in Table 1 in ascending order of survival period (4° storage). Of the bacteria preserved

TABLE 1

Cultural conditions used and survival period of bacteria preserved on anhydrous silica gel stored at 4° and room temperature

Identification	Culture collection* and strain	Culture medium† and incubation temperature (°)	Survival period (weeks)‡ during storage at 4°	Survival period (weeks)‡ during storage at Room temp.
No viable bacteria at subsequent testing				
Chromobacterium marismortui	NCIB 8731	PSG, 30	(5)	(9)
Erwinia salicis	NCPPB 1466	NA, 30	(5)	(5)
Pseudomonas fragi	NCDO 752	NA, 25	(9)	(9)
Rhizobium trifolii	UCS RES	NA, 25	(9)	(5)
Alcaligenes metalcaligenes	NCIB 8734	NA, 25	(23)§	(3)§
Salmonella typhimurium	SL 516	NA, 37		(17)
Yersinia rodentium	NCTC 8315	ENA, 30	(38)	(26)
Escherichia coli	NCIB 8277	NA, 37	(45)	(1)
Shigella sonnei	NCTC 8220	NA, 37	(49)	
Staphylococcus aureus	NCIB 8244	NA, 37	(54)	(27)
Proteus vulgaris	NCTC 4636	NA, 30	(62)	(54)
Nocardia sp.	NCIB 8862	NA, 25	(103)	(57)
Serratia marcescens	NCTC 8706	NA, 25	(108)	(62)
Viable bacteria at last testing (4° storage)				
Bacillus stearothermophilus	NCIB 8157	NA, 45	142	142
Cellulomonas flavigena	NCIB 8073	NA, 30	142	(59)
Microbacterium lacticum	NCDO 747	NA, 30	142	(59)§
Mycobacterium flavum	NCIB 10071	NA, 30	142	(59)§
Mycobacterium smegmatis	NCTC 333	ENA, 30	142	142
Nocardia calcarea	NCIB 8863	NA, 25	142	(59)§
Nocardia petroleophila	NCIB 9438	NA, 25	142	
Mycobacterium phlei	NCTC 8151	ENA, 30	146	(59)
Staphylococcus epidermis	NCIB 8558	NA, 37	163	(80)
Proteus vulgaris	NCTC 8313	NA, 30	166§	(30)
Salmonella typhimurium	UCS WT	NA, 37	184	(55)
Arthrobacter globiformis	NCIB 8717	NA, 25	186	186
Arthrobacter ureafaciens	NCIB 7811	NA, 30	186	186
Mycobacterium rhodochrous	NCIB 8727	NA, 30	186	(103)
Nocardia cellulans	NCIB 8868	NA, 25	186	(53)
Sarcina lutea	NCIB 8553	NA, 25	188	185
Bacillus subtilis	NCIB 3610	NA, 30	190	190
Klebsiella aerogenes	NCTC 8172	NA, 30	191	(108)
Bacillus megaterium	NCDO 685	NA, 30	194	191
Micrococcus flavus	NCIB 8166	NA, 25	194	191

* NCDO, National Collection of Dairy Organisms; NCIB, National Collection of Industrial Bacteria; NCPPB, National Collection of Plant Pathogenic Bacteria; NCTC, National Collection of Type Cultures; SL, Professor B. Stocker, Lister Institute, London; UCS, University College Swansea, Departmental Collection.

† Media. NA, Nutrient Agar (Oxoid); ENA, NA+glucose (1% w/v), tryptone (Oxoid, 1% w/v), yeast extract (Oxoid, 1% w/v); PSG, Halophile agar medium; Payne, Sehgal & Gibbons (1960).

‡ Weeks: survival period at last testing; (weeks): survival period, no viable micro-organisms at subsequent testing.

§ Survival period, marked reduction in microbial numbers and/or increased lag period.

on silica gel and stored at room temperature, 24% were still viable at the last testing and under these conditions the limit of survival was at least 4 years in the case of 6 organisms. A further 40% of the bacteria tested remained viable for at least one year. Storage at 4° increased the survival period 2- to 3-fold in many instances. 60% of the bacteria were still viable at the last testing and a further 12% survived at least 1 year at 4°. Of the 12 strains which did not survive the testing period although stored at 4°, 10 were Gram negative bacilli. However, 3 other Gram negative bacilli were still viable at the last testing.

The 22 different fungi which survived dehydration on silica gel are listed in Table 2. Of the fungi stored at room temperature, 68% were still viable at the latest testing

TABLE 2

Cultural conditions used and survival period of fungi preserved on anhydrous silica gel stored at 4° and room temperature

Identification	Culture collection* and strain	Culture medium† and incubation temperature (°)	Survival period (weeks)‡ during storage at 4°	Survival period (weeks)‡ during storage at Room temp.
No viable fungi at subsequent testing				
Penicillium terrestre	CMI 89385	CD, 25	(101)	(47)§
Viable fungi at last testing				
Gliocladium sp.	II ARGT	CD, 25		34§
Mucor hiemalis	II cmi+	CD, 25		34
Mucor hiemalis	II cmi−	CD, 25		34
Verticillium albo-atrum	II potato	CD, 25	52‖	(52)
Thamnidium elegans	II cmi	CD, 25		61
Verticillium albo-atrum	II lucerne	CD, 25		61
Verticillium albo-atrum	II 4ES	CD, 25		61
Verticillium lateritium	II soil	CD, 25		61
Fusarium oxysporum	II pea	CD, 25	52‖	113
Verticillium nigrescens	II potato	CD, 25		114§
Verticillium dahliae	II pea	CD, 25	52‖	117§
Saccharomyces cerevisiae	NCYC 24	ENA, 25	142	(12)
Saccharomyces carlsbergensis	NCYC 73	ENA, 25	146§	(12)
Verticillium nubilum	II potato	CD, 25		157§
Verticillium tricorpus	II tomato	CD, 25		157
Penicillium sp.	UCS C8	CD, 25	190	(55)
Penicillium sp.	UCS C9	CD, 25	190	(101)
Penicillium sp.	UCS CA	PDA, 25	190	190
Penicillium sp.	UCS ZW101	CD, 25	190	190§
Sordaria fimicola	UCS 7+	PDA, 25	190§	(57)
Aspergillus niger	UCS	CD, 25	191	191

* CMI, Commonwealth Mycological Institute; II, Professor Ivor Isaac, University College of Swansea; NCYC, National Collection of Yeast Cultures; UCS, University College of Swansea, Departmental Collection.

† Media. ENA, Nutrient Agar (Oxoid)+glucose (1% w/v), tryptone (Oxoid, 1% w/v); yeast extract (Oxoid, 1% w/v); CD, Czapek Dox Agar, modified (Oxoid); PDA, Potato Dextrose Agar (Oxoid).

‡ Weeks, survival period at last testing; (weeks), survival period, no viable micro-organisms at subsequent testing.

§ Survival period, marked reduction in microbial numbers and/or increased lag period.

‖ Rogers (1972).

and under these conditions of storage the limit of survival was at least 3 years for 5 fungi. A total of 77% of the fungi tested remained viable for 1 year or more with storage at room temperature. A comparison between room temperature storage and 4° storage can be made for 6 fungi; the minimum survival period at room temperature was always shorter. Six of the fungi (27%) survived at 4° for the duration of the testing period (nearly 4 years) and the 2 yeasts were still viable at their latest testing (nearly 3 years at 4°).

Silica gel preparations additional to those in Tables 1 and 2 were in paired tubes having either cotton-wool plugs or screw-caps. In 4 cases the latter tubes exhibited an increase in survival period which was *c.* 2- to 4-fold. In one case the screw-capped tube showed a decrease in survival period and with 7 pairs no differences were revealed at the last testing.

Discussion

The results from successful preservation on silica gel confirm the validity of the preservation method and extend considerably the range of bacteria and fungi to which this method may be applied. These results also demonstrate the beneficial effect of storage at low temperature. A comparison exists for 39 microbial strains; only with *Chromobacterium marismortui* (Table 1) was the viability at 4° (viable at 5 weeks, non-viable at 9 weeks) lost earlier than at room temperature (viable at 9 weeks but not subsequently). However, for specified bacteria or fungi, long-term storage on silica gel at room temperature is possible if refrigeration is not available. For the majority of the micro-organisms studied as silica gel preparations, short-term exposure to ambient temperature (e.g. postal despatch) is not ruled out by the present results.

Different microbial groups are known to respond differently to established preservation methods. This may account for the failures using the silica gel method reported for streptomyces (Hopwood & Ferguson, 1969; Kutzner, 1972), flexibacteria (Sanfilippo & Lewin, 1970) and some failures recorded by Grivell & Jackson (1969). The results of the present investigation suggest that Gram positive bacilli tend to survive preservation on silica gel better than most Gram negative bacilli. A similar tendency is reported for survival after drying *in vacuo* (Miller & Simons, 1962) and following lyophilization (Floodgate & Hayes, 1961; Steel & Ross, 1963). The latter phenomenon is influenced by many factors (e.g. cultural conditions, age at harvest, suspending medium). These factors also influence survival after preservation on silica gel (D. R. Trollope, unpublished) and in particular may account for recorded variations in the survival period of *E. coli* using the technique of Perkins (1962). Brown (1971), using 63 strains, reported 100% viability up to 3 years after preservation and Grivell & Jackson (1969) indicated 45–66 weeks of successful preservation at the latest testing of their 6 strains. However, Reinhardt (1966) reported that *E. coli* (included as food for *Acr. rosea*) did not survive 26 weeks and in the present study, viable *E. coli* were recovered at 45 but not at 54 weeks. Another factor found to influence survival following lyophilization is residual moisture and Perkins (1962) suggested that it was an important factor for silica gel preservation. This suggestion is supported by the present results using paired tubes which favour the use of a closed tube.

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