# Viability of lyophilized fungal cultures

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Results of a viability test on 17–18 years old lyophilized cultures are reported.

Freeze-drying as a means of prolonged preservation of fungal strains has been applied at the CBS since 1958. Theoretical knowledge derived from Raper and Alexander (1945) and Harris (1954) was initially applied and later the work was stimulated by reports of viability tests and the occasional revival of CBS lyophilized cultures.

Viability checks on lyophilized fungal cultures were published by Raper and Alexander (1945) up to 40 months after processing; by Mehrota and Hesseltine (1958) on cultures up to 15 years in age; by Hesseltine, Bradle and Benjamin (1960) on cultures up to 17 years in age; by Ellis and Roberson (1968) on cultures up to 23 years in age. Recently Sarbhoy and al. (1974) reported their experiences with 1–2 years of freeze-drying.

In 1976 it seemed desirable to test a number of the oldest lyophilized CBS strains for viability.

A hundred of the 17–18 years old lyophilized cultures that survived the initial treatment, were tested. As the majority of revived cultures in literature are of species in the genera *Penicillium* and *Aspergillus*, only a few of these are included.

Each pellet (spores + skim milk) was dissolved in approx. 2 ml sterile water, poured onto petridishes containing a medium favourable for the species concerned and routinely incubated at 25 C. Where required, cultures were grown at different temperatures and/or in light (daylight, black light or otherwise).

Results of this viability test on 17–18 years old lyophilized cultures are listed in Table 1. Of the 100 cultures tested, 91 were viable. Of those not viable, the strains marked with an asterisk had suffered a poor vacuum during processing. Pressure during processing, measured with a McLeod gauge, should preferably be less than 0.1 mg Hg. Experience has taught us that in some genera (e.g. *Mucor*, *Rhizopus*, *Streptomyces* etc.) spores seem to loose the ability to germinate within a relatively short period compared with other genera and thus subcultures should be young at the time of processing. Osmophilic species

Table 1. Results of viability test on 17–18 years old lyophilized cultures.

| CBS no. | Name | Viability |
|---|---|---|
| 130.51 | *Acremonium apii* (M. A. Smith et Ramsey) W. Gams | + |
| 122.42 | *Acremonium furcatum* (F. et V. Moreau) ex W. Gams | – |
| 124.26 | *Acremonium kiliense* Grütz | + |
| 122.29 | *Acremonium kiliense* Grütz | + |
| 123.29 | *Acremonium kiliense* Grütz | + |
| 135.35 | *Acremonium kiliense* Grütz | + |
| 139.37 | *Acremonium kiliense* Grütz | + |
| 137.35 | *Acremonium recifei* (Leão et Lôbo) W. Gams | –* |
| 134.58 | *Acremonium sclerotigenum* (F. et V. Moreau ex Valenta) W. Gams | + |
| 107.58 | *Actinoplanes philippinensis* Couch | + |
| 109.58 | *Alternaria radicina* Meier, Drechsler et Eddy | – |
| 110.58 | *Alternaria radicina* Meier, Drechsler et Eddy var. *petroselini* (Neergaard) Neergaard | + |
| 110.58 | *Anixiopsis fulvescens* (Cooke) de Vries var. *stercoraria* (Hansen) de Vries | + |
| 315.58 | *Apiosordaria verruculosa* (Jensen) v. Arx et W. Gams | + |
| 105.55 | *Aspergillus aureolus* Fennell et Raper | + |
| 109.55 | *Aspergillus deflectus* Fennell et Raper | + |
| 114.39 | *Aspergillus niger* van Tieghem | + |
| 126.28 | *Aspergillus sejunctus* Bain. et Sartory | + |
| 306.58 | *Botryosphaeria rhodina* (Berk. et Curt.) v. Arx | – |
| 124.58 | *Botrytis cinerea* Pers. ex Fr. | + |
| 126.58 | *Botrytis cinerea* Pers. ex Fr. | + |
| 113.24 | *Centrospora acerina* (Hartig) Newhall | –* |
| 149.52 | *Cephalosporium deformans* Crandall | + |
| 115.47 | *Ceratocystis ulmi* (Buisman) C. Moreau | + |
| 137.58 | *Chaetomium angustispirale* Sergejeva | + |
| 156.52 | *Chaetomium cuniculorum* Fuckel | + |
| 148.51 | *Chaetomium globosum* Kunze ex Fr. | + |
| 141.58 | *Chaetomium perlucidum* Sergejeva | + |
| 122.57 | *Chaetomium quadrangulatum* Chivers | + |
| 143.58 | *Chaetomium semen-citrulli* Sergejeva | + |
| 144.58 | *Chaetomium simile* Massee et Salmon | + |
| 147.50 | *Chrysosporium luteum* (Cost.) Carmichael | + |
| 164.58 | *Coemansia aciculifera* Linder | + |
| 165.58 | *Coemansia aciculifera* Linder | + |
| 181.49 | *Coprinus myceliocephalis* M. Lange | + |
| 177.58 | *Curvularia pallescens* Boedijn | + |
| 190.48 | *Curvularia trifolii* (Kauffman) Boedijn | + |
| 239.51 | *Dictyostelium discoideum* K. B. Raper | –* |
| 224.58 | *Drechslera catenaria* (Drechsler) S. Ito | –* |
| 107.07 | *Fusarium equiseti* (Corda) Sacc. | + |
| 178.29 | *Fusarium oxysporum* Schlecht ex Fr. | + |
| 179.29 | *Fusarium oxysporum* Schlecht ex Fr. f. *medicaginis* Schlecht ex Fr. (Weimer) Snyder et Hansen | + |
| 181.29 | *Fusarium solani* (Mart.) Appel et Wollenw. | + |
| 137.33 | *Fusarium tabacinum* (van Beyma) W. Gams | + |
| 275 50 | *Gelasinospora reticulispora* (Greis et Greis-Dengler) C. et M. Moreau | + |
| 229.58 | *Isaria felina* (DC.) ex Fr. | + |
| 185.57 | *Leptosphaeria nodorum* E. Müller | + |
| 240.58 | *Microascus desmosporus* (Lechmere) Curzi | + |
| 242.58 | *Monascus ruber* van Tieghem | + |
| 246.58 | *Mucor plumbeus* Bon. | + |

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|---|---|---|
| 259.47 | *Neurospora crassa* Shear et B. O. Dodge | + |
| 255.58 | *Nocardia paraffinae* (Jensen) Waksman et Henrici | + |
| 267.35 | *Penicillium aurantio-violaceum* Biourge | + |
| 297.58 | *Petriella asymmetrica* Curzi | + |
| 141.41 | *Phialophora malorum* (Kidd et Beaumont) Mc.Colloch | + |
| 142.41 | *Phialophora mustea* Neergaard | + |
| 272.31 | *Pilobolus crystallinus* (Wiggers) Tode | + |
| 213.57 | *Piptocephalis virginiana* Leadbeater et Mercer | + |
| 322.58 | *Pseudeurotium zonatum* van Beyma | + |
| 285.47 | *Rhinocladiella compacta* (Carion) Schol- Schwarz | + |
| 296.31 | *Rhizopus arrhizus* Fischer | + |
| 285.55 | *Rhizopus arrhizus* Fischer | + |
| 256.28 | *Rhizopus chinensis* Saitô | + |
| 327.47 | *Rhizopus delemar* (Boidin) Wehmer et Hanzawa | + |
| 347.49 | *Rhizopus stolonifer* (Ehrenb. ex Fr.) Lind | + |
| 405.51 | *Rhizopus thermosus* Yamamoto | + |
| 128.08 | *Rhizopus tritici* Saitô | + |
| 304.56 | *Shanorella spirotricha* R. K. Benjamin | + |
| 305.56 | *Shanorella spirotricha* R. K. Benjamin | + |
| 404.59 | *Sporormia minima* Auersw. | + |
| 364.58 | *Staphylotrichum coccosporum* Meyer et Nicot | + |
| 102.58 | *Streptomyces diastaticus* (Krainsky) Waksman et Henrici | + |
| 104.58 | *Streptomyces diastaticus* (Krainsky) Waksman et Henrici | + |
| 410.58 | *Streptomyces diastaticus* (Krainsky) Waksman et Henrici | + |
| 100.56 | *Streptomyces diastaticus* (Krainsky) Waksman et Henrici var. *ardesiacus* Baldacci et al. | + |
| 700.57 | *Streptomyces natalensis* Struyk et al. | + |
| 240.57 | *Streptomyces noursei* Brown et al. in Hazen et Brown | + |
| 437.51 | *Streptomyces rimosus* Waksman in Waksman et Lechevalier | + |
| 410.52 | *Streptomyces viridichromogenus*(Krainsky) Waksman et Henrici | + |
| 105.58 | *Streptomyces viridIdiasticus* (Baldacci et al. ) Pridham et al. | + |
| 106.58 | *Streptomyces viridIdiasticus* (Baldacci et al.) Pridham et al. | + |
| 415.54 | *Syncephalis aurantiaca* Vuill. | + |
| 376.58 | *Syncephalis cornu* van Tieghem et le Monnier | + |
| 416.54 | *Syncephalis nodosa* van Tieghem | + |
| 379.58 | *Torula herbarum* Link ex Fr. | + |
| 429.58 | *Tricellula curvata* Haskins | + |
| 370.52 | *Trichoderma harzianum* Rifai | + |
| 126.27 | *Verticillium lecanii* (Zimm.) Viégas | + |
| 383.35 | *Verticillium lecanii* (Zimm.) Viégas | + |
| 123.42 | *Verticillium lecanii* (Zimm.) Viégas | + |
| 396.58 | *Verticillium psalliotae* Treschow | + |
| 290.30 | *Verticillium serrae* (Maffai) van Beyma | + |
| 400.58 | *Volutella colletotrichoides* Chilton | + |
| 379.55 | *Westerdijkella ornata* Stolk | + |
| 469.59 | *Xeromyces bisporus* Fraser | – |
| 402.58 | *Zygorhynchus californiensis* Hesseltine et al. | + |
| 403.58 | *Zygorhynchus exponens* Burgeff | + |
| 404.58 | *Zygorhynchus exponens* Burgeff var. *smithii* Hesseltine et al. | + |
| 405.58 | *Zygorhynchus heterogamus* (Vuill.) Vuill. | + |
| 406.58 | *Zygorhynchus moelleri* Vuill. | + |

+ : growth.
– : no growth.
* : poor vacuum during processing.

(e.g. *Xeromyces bisporus* = *Monascus bisporus*) grown on media with a high sugar content never form compact pellets, probably due to poor freezing. Species which normally sporulate poorly in vitro or only produce spores at an advanced age (e.g. *Botryosphaeria* spp., *Centrospora* spp.) either do not survive lyophilization, or produce very few colonies. It is unlikely that the latter species would still have viable spores after a number of years.

Revived cultures mostly showed better sporulation than those which had been periodically transferred since 1958.

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