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Preservation of fungi in water (Castellani): 20 years

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Abstract

Five-hundred ninety-four strains of fungi were studied. They were found being preserved with Castellani's method with distilled water during 1 to 20 years. 62% of the strains (n = 368) did grow when subcultured and maintained their main morphological features. 90% of the 20 years old strains of different species were viable. It is argued that the technique of introduction of the strains into the water and their optimal condition will determine survival. The Castellani's method is recommended as easy, cheap and satisfactory for preservation of most species of fungi.

Introduction

A living culture collection of fungi that states their genetic stability, pathogenicity, purity and viability should be an important part of a mycological laboratory, as a reference for the diagnosis of mycoses that affect man and animals, in comparative and taxonomic studies of isolates and in the training of mycology students and allied personnel.

The most suitable methods of conservation currently used in the biggest collections (C.B.S., A.T.C.C., N.C.T.C., etc.) seems to be that of freeze-drying (lyophilization) [3, 8, 18, 19, 25, 27, 29] along with preservation in liquid nitrogen under mineral oil and/or periodic subculturing.

There are other methods [1, 3, 7, 9, 10, 24, 26, 28, 32] which are very laborious and expensive that can hardly be applied in underdeveloped countries due to economic difficulties.

A very useful technique is that of Castellani [13], published four decades ago, which seems to be the most suitable for small collections with little funding [2, 22, 20].

In the few reported experiences with this method [2, 12, 15, 16, 20, 23, 33] it has been shown a range of survival between two and three years of those fungal strains being annually subcultured.

Since 1966 a modified version of Castellani's method [30] has been used in the medical mycology department of the Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas. According to this method, the pieces of fungal cultures are introduced in screw cap tubes with sterile distilled water and left at room temperature (25–28 °C).

The present study reports the morphological stability, purity and viability of 594 strains which were found preserved in water for a period between 1 to 20 years.

Material and methods

Biological material

One-hundred-sixteen species represented in 594 strains were chosen randomly from the fungal collection in the mycology department, Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas. The strains were found preserved for a period between 1–20 years according to Castellani's modified method [30] (fungal cultures submerged in distilled water in screw cap tubes).

Culture media

Lactritmel, Sablac, Sablac without antibiotics [5]. All these media are based on milk and other natural products, used also in the first isolation and description of the strains.

Subculturing from the water

1. Cleaning of the bakelit screw caps with ethyl-alcohol (70°), before unscrewing them.
2. from each sample a fragment was transferred to each culture media (2 tubes).
3. Incubation of the strains at laboratory temperature (24–28 °C) between 5–30 days, if the strain did not grew in this period the process was repeated.
4. Identification was confirmed by slide cultures [6] and the morphological features compared with the first description of the strain.

Results

The number of strains grouped by species and age is variable, all the results are assembled in Table I.

Viability of strains:

- 62% (n = 368) of the 594 studied strains were found viable and their main morphological features corresponded to the original description (Table 1).

Viability of species:

- 73.5% (n = 116) had a viability of 50% (or more) maintained their original features (Table 1).

Viability and age:

- In almost every year there was a survival rate of over 50% (or more) of the strains (Fig. 1).

Contamination:

- 22.8% (n = 135) of all the strains (n = 594) were found contaminated and only 32.5% (n = 44) could be reisolated from the contaminated cultures.
- The contaminants were basically other fungi (*Aspergillus* sp., *Penicillium* sp. and *Cladosporium* sp.).

Discussion

One-hundred-sixteen different species were studied encompassing an universe of 594 strains, which were found preserved in water for a period between 1 to 20 years. Sixty-two percent (n = 368) of all the strains (n = 594) were viable and mantained their main morphological features. Of the 20 years old strains (n = 19) 90% were still alive. The 20 years old species that survived did belong to different species: *Candida guillermondii, Fusarium falciforme* as *Cephalosporium falciforme, Cladosporium carrionii, Diplorhinothricum gallopavonum, Endomycopsis chodatii, Fonsecaea compacta, Madurella mycetomatis* as *Madurella mycetomi, Phialophora gougerotii* as *Exophiala gougerotii, Phialophora verrucosa, Trichosporon behrendii, Trichophyton tonsurans, Wangiella dermatitidis, Cladosporium bantianum* as Xylophypha bantiana.

Table 1.

	Genera and species	Strains	Living strain %	Life-span (years)
1	*Acrotheca aquaspersa*	2 (1–2)	50	7
2	*Actinomyces paraguayensis*	2 (3–4)	0	–
3	*Alternaria* sp.	2 (5–6)	0	–
4	*Arthroderma gloriae*	1 (7)	0	–
5	*Arthroderma rosum*	2 (8–9)	100	10–18
6	*Arthroderma simii*	2 (10–11)	50	19
7	*Arthroderma tuberculatum*	2 (12–13)	50	1
8	*Aspergillus fumigatus*	1 (14)	100	3
9	*Aspergillus niger*	1 (15)	100	19
10	*Aspergillus terreus*	3 (16–18)	100	1–14
11	*Beauveria* sp.	1 (19)	0	–
12	*Blastomyces dermatitidis*	9 (20–28)	55.5	1–14
13	*Candida albicans*	2 (29–30)	50	3
14	*Candida guillermondii*	1 (31)	100	20
15	*Candida krusei*	1 (32)	0	–
16	*Candida tropicalis*	1 (33)	100	19
17	*Cephalosporium falciforme*	1 (34)	100	20
18	*Chmelia slovaca*	4 (35–38)	50	1
19	*Chrysosporium keratinophilicum*	7 (39–45)	57.1	1–18
20	*Chrysosporium* sp.	4 (46–49)	100	1–18
21	*Chrysosporium tropicum*	7 (50–56)	85.7	1–18
22	*Cladophialophora ajelloi*	2 (57–58)	50	10
23	*Cladosporium castroi*	2 (59–60)	50	2
24	*Cladosporium carrionii*	32 (61–92)	75	1–20
25	*Cladosporium castellanii*	10 (93–102)	80	3–10
26	*Cladosporium devriesii*	1 (103)	100	1
27	*Cladosporium mansonii*	1 (104)	100	1
28	*Cladosporium resinae*	2 (105–106)	50	14
29	*Cladosporium* sp.	2 (107–108)	50	2
30	*Cryptococcus diffluens*	2 (109–110)	50	7
31	*Cryptococcus neoformans*	8 (111–118)	50	2–18
32	*Dactylaria funiculata*	3 (119–121)	0	–
33	*Diplorhinothricum gallopavonum*	3 (122–124)	66.7	11–20
34	*Endomycopsis chodatii*	3 (125–127)	33.4	20
35	*Endomycopsis fibuligera*	5 (128–132)	25	3
36	*Entomophthora coronata*	1 (133)	100	19
37	*Epidermophyton floccosum*	4 (134–137)	50	3
38	*Exophiala spinifera*	2 (138–139)	100	19
39	*Exophiala jeanselmei*	8 (140–147)	37.5	1–10
40	*Exphiala salmonis*	2 (148–149)	0	–
41	*Exophiala* sp.	2 (150–151)	50	1
42	*Fonsecaea compacta*	7 (152–158)	71.4	9–20
43	*Fonsecaea pedrosoi*	58 (159–216)	77.5	1–13
44	*Fusarium solani*	10 (217–226)	100	1–5
45	*Hendersonula toruloidea*	7 (227–233)	57.1	1–2
46	*Histoplasma capsulatum*	4 (234–237)	50	5–10
47	*Hyalopus* sp.	5 (238–242)	80	1–18
48	*Leptosphaeria senegalensis*	2 (243–244)	0	–
49	*Loboa loboi*	2 (245–246)	0	–
50	*Madurella grisea*	30 (247–276)	73.4	1–19
51	*Madurella mycetomi*	6 (277–282)	50	1–20

Table 1. (continued).

	Genera and species	Strains	Living strain %	Life-span (years)
52	*Margarinomyces* sp.	2 (283–284)	100	1
53	*Microsporum amazonicum*	2 (285–286)	50	18
54	*Microsporum audouinii*	2 (287–288)	50	11
55	*Microsporum boullardii*	1 (289)	100	3
56	*Microsporum canis*	10 (290–299)	70	1
57	*Microsporum cookei*	1 (300)	100	18
58	*Microsporum ferrugineum*	6 (301–306)	50	1–19
59	*Microsporum fulvum*	1 (307)	100	18
60	*Microsporum gallinae*	3 (308–310)	66.7	1–7
61	*Microsporum gypseum*	2 (311–312)	100	9–18
62	*Microsporum racemosum*	4 (313–316)	100	10–14
63	*Microsporum rivalieri*	1 (317)	0	–
64	*Microsporum vanbreuseghemii*	1 (318)	0	–
65	*Monosporium apiospermium*	2 (319–320)	100	1–2
66	*Nannizzia gypsea*	5 (321–325)	60	3–9
67	*Neotestudina rosatii*	1 (326)	0	–
68	*Nocardia asteroides*	6 (327–332)	83.4	1–19
69	*Nocardia brasiliensis*	10 (333–342)	50	1–19
70	*Nocardia farcinica*	1 (343)	0	–
71	*Nocardia mexicana*	1 (344)	0	–
72	*Nocardia rhodnii*	2 (345–346)	50	5
73	*Nocardia* sp.	2 (347–348)	100	2–5
74	*Penicillium* sp.	1 (349)	100	2
75	*Petriella sordida*	1 (350)	100	11
76	*Petriellidium boydii*	9 (351–359)	100	1–19
77	*Phaeoannellomyces werneckii*	33 (360–392)	63.7	1–19
78	*Phialophora citrina*	1 (393)	100	1
79	*Phialophora gougerotii*	5 (394–398)	60	1–20
80	*Phialophora richardsiae*	1 (399)	100	2
81	*Phialophora spinifera*	7 (400–406)	85.7	1–12
82	*Phialophora verrucosa*	18 (407–424)	66.7	1–20
83	*Pichia delftensis*	2 (425–426)	50	3
84	*Piedraia hortae*	2 (427–428)	0	–
85	*Plenodomus avramii*	2 (429–430)	0	–
86	*Plenodomus variabilis*	3 (431–433)	0	–
87	*Pseudochaetosphaeronema larense*	7 (434–440)	42.8	1–12
88	*Pyrenochaeta mackinonii*	5 (441–445)	80	1–15
89	*Pyrenochaeta romeroi*	3 (446–448)	33.4	11
90	*Pyrenochaeta* sp.	2 (449–450)	50	10
91	*Ramichloridium cerophilum*	2 (451–452)	100	1–2
92	*Rhinocladiella aquaspersa*	8 (453–460)	62.5	1–2
93	*Rhinocladiella atrovirens*	3 (461–463)	66.7	1–11
94	*Rhinocladiella* sp.	7 (464–470)	100	8–19
95	*Sporothrix schenckii*	15 (471–485)	73.4	3–13
96	*Stenella araguata*	9 (486–494)	33.4	1–11
97	*Streptomyces madurae*	2 (495–496)	0	–
98	*Streptomyces pelletieri*	2 (497–498)	0	–
99	*Streptomyces somaliensis*	2 (499–500)	0	–
100	*Torula bergeri*	1 (501)	0	–
101	*Torula poikilospora*	4 (502–505)	100	1–12
102	*Trichophyton gallinae*	3 (506–508)	66.7	10–19

Table 1. (continued).

	Genera and species	Strains	Living strain %	Life-span (years)
103	*Trichophyton megninii*	1 (509)	100	12
104	*Trichophyton mentagrophytes*	9 (510–518)	88.9	1–19
105	*Trichophyton phaseoliforme*	3 (519–521)	33.4	12
106	*Trichophyton rubrum*	4 (522–525)	50	13
107	*Trichophyton schoenleinii*	2 (526–527)	50	–
108	*Trichophyton simii*	1 (528)	100	11
109	*Trichophyton soudanense*	2 (529–530)	50	11
110	*Trichophyton* sp.	1 (531)	0	–
111	*Trichophyton tonsurans*	1 (532)	100	20
112	*Trichophyton verrucossum*	1 (533)	100	11
113	*Trichophyton violaceum*	1 (534)	0	–
114	*Trichosporon behrendii*	6 (535–540)	100	19–20
115	*Wangiella dermatitidis*	38 (541–578)	52.6	1–20
116	*Xylohypha bantiana*	16 (579–594)	43.7	1–20

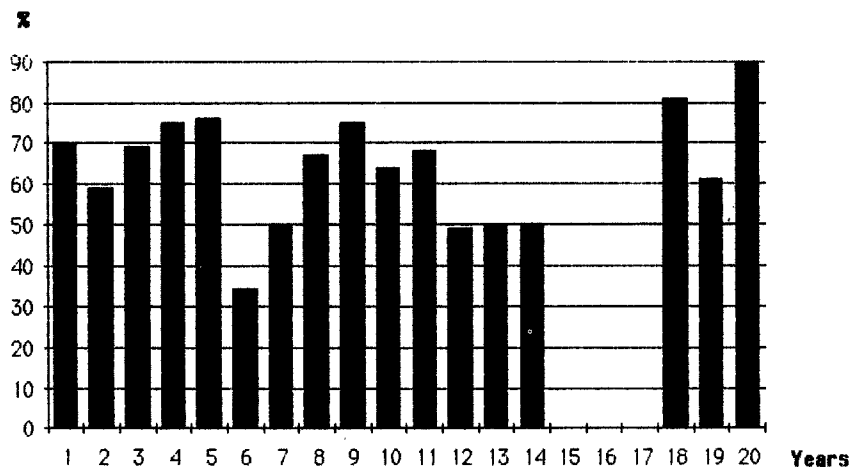

Fig. 1. Yearly survival rate.

In relation to the strains of the same species, over 50% were viable as well as in each year there was a survival rate of over 50%. All this suggest that the conditions of a given strain and not its species or genus were determinant for survival. A *Trichophyton tonsurans* strain was perfectly viable and not pleomorphic after 20 years. One could speculate that the survival rate is higher but there were limitations as regards the technique of preparation of the specimen for preservation.

Thirty-eight percent of the strains were found dead. It could not be precised the actual time when they died as there was no control of survival of the period in between. The number of strains by species and age is variable therefore it is impossible to establish a significative statistical relationship between species, viability and age. It could be argued that it is the technique of introduction of the strain into the water and its optimal condition that will determine its survival, because the conditions in the tube should change little after some time. So, a strain that survives 5 years is most likely to survive 20 years as well, it is like being in a hibernation state.

The survival rate for the conservation of fungi with the method of lyophilization is higher

[18, 27]. But the Castellani method is extremely easy, cheap and satisfactory for the preservation of most species and it should be taken in mind as a method of preservation together with the standard ones (lyophilization, etc.) in any laboratory.

The Castellani's method seems to be the elective one for those laboratories that don't have enough funding and dotation.

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