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STORAGE OF LYOPHILIZED DERMATOPHYTE CULTURES

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Abstract

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Viability of lyophilized fungus cultures was tested after one year storage at a temperature of 2 to 6 °C. In 10 of 13 lyophilized cultures a slight but non-significant decrease in viability occurred. Only for 3 lyophilized strains stored in a refrigerator less than 78 % of the fungus units were viable when compared with original values found immediately after lyophilization. This decrease was significant. Viability of all lyophilized cultures stored at room temperature decreased significantly or highly significantly with no differences between samples stored in the dark or light.

Viability, refrigerator, room temperature, dark, light.

Lyophilization has been successfully employed for long-term storage of various microorganism cultures. It is of great importance also in mycology. Lyophilized cultures of fungus retain their vital properties for years without demonstrating pleiomorphism or other changes. Thus strain properties remain unchanged, a fact which creates advantages in the field of biotechnology. However, experiments have shown that for some fungal cultures this mode of storage is not suitable. Among such fungi are also numerous strains of dermatophytes. For these dermatophyte cultures the best survival following lyophilization was found in those which formed large numbers of microconidia (Nikiforov 1976). Viability of cultures forming hyphae, chlamydospores and macroconidia was clearly decreased after lyophilization (Rybnikář et al. 1983). The numbers of organisms surviving the lyophilization procedure is dependent not only on micromorphological features of the fungus in culture, but also on further factors such as selection of a suitable lyophilization medium and parameters used in the specific steps of lyophilization.

A decrease in viability of fungus elements occurs during their storage in lyophilized form. Along with residual moisture further physical factors such as storage temperature and light may be involved. Despite the fact that long-term storage of lyophilized fungi is usually at refrigerator temperatures (Ellis and Roberson 1968; Wawrzkiewicz 1976 and others) good viability of lyophilized pathogenic fungi has been reported after 10 years storage at room temperature (Bosmans 1974). These reports prompted us to investigate the possibility of storing lyophilized cultures of dermatophytes at room temperature under both light and dark conditions. We compared quantitative results on survival of lyophilized dermatophytes after one year of storage under the above-mentioned conditions contrasted with refrigerator storage.

Materials and Methods

The vaccination strain of *Trichophyton verrucosum* CCM E-650 (3 experimental batches) and another 10 strains of dermatophytes (see Table 1) were used in the experiment. Their multiplication and lyophilization were carried out as described earlier (Rybnikář 1981). For lyophilization medium a solution of gelatine and saccharose was used.

Immediately after the lyophilization procedure had been finished samples of each strain were tested for viability using the common plate dilution method (for details see Rybnikář 1981). The remaining lyophilized samples (lyophilizates in vacuum) were kept at room temperature (18 to 26 °C) for one year under both light and dark conditions, and at refrigerator temperature (2 to 6 °C) in the dark. After this period again the viability of organisms was tested using the above-mentioned method. The results of this test were compared with those obtained immediately

Table 1

Survival of lyophilized dermatophyte cultures after one year storage at refrigerator and room temperatures in the light and inthe dark
Values designated × differ significantly from original values found immediately after lyophilization. P=0,01 × × ; P=0,05 × ; n=14

Lyophilized culture	No. of viable organisms/cm³ ($x \pm S_{\bar{x}}$)				Per cent of surviving fungal elements after 1 year storage at temperature:		
	Immediately after lyophilization	1 year after storage at temperature:					
		refrigerator	room dark	room light	refrigerator	room dark	room light
Epidermophyton floccosum	71.300 ± 6.100	59.250 ± 5.350	× ×32.250 ± 3.190	× × 36.250 ± 3.830	83.1	45.2	50.8
Microsporum canis No. 4043	4.470 ± 830	× 2.200 ± 270	× × 510 ± 66	× × 770 ± 59	49.2	11.4	17.2
Microsporum canis No. 6602	2.280 ± 380	2.700 ± 480	× × 1.030 ± 160	× × 1.360 ± 140	95.7	36.5	48.2
Microsporum cookei	24.000 ± 2.160	23.470 ± 2.350	× × 6.900 ± 600	× × 8.900 ± 800	97.8	28.8	37.1
Microsporum gypseum	8,800.000 ± 630.000	7,800.000 ± 870.000	× × 1,475,000 ± 136.000	× × 1,778.000 ± 210.000	88.6	16.8	20.2
Trichophyton ajelloi	109.000 ± 12.700	× × 61.000 ± 5.500	× × 28.200 ± 1.600	× × 21.500 ± 1.700	56.0	25.9	19.7
Trichophyton equinum	14,475.000 ± 686.000	× ×9,800.000 ± 718.000	× × 7,325.000 ± 414.000	× × 6,475.000 ± 680.000	67.7	50.6	44.7
Trichophyton mentagrophytes No. 506	3,090.000 ± 316.000	2,670.000 ± 136.000	× 2,045.000 ± 326.000	×1,960.000 ± 266.000	86.4	66.2	63.4
Trichophyton mentagrophytes No. 509	29,300.000 ± 3,800.000	29,000.000 ± 2,500.000	× × 19,975.000 ± 1,690.000	× × 17,950.000 ± 1,870.000	99.0	68.2	61.3
T. mentagrophytes var. quinckeanum	64,010.000 ± 4,460.000	57,000.000 ± 5,150.000	× 47,750.000 ± 4,840.000	× 43,250.000 ± 6,450.000	89.0	74.6	67.6
Trichophyton verrucosum, batch 1	21,800.000 ± 2,005.000	17,133.000 ± 1,320.000	× × 11,000.000 ± 1,210.000	× × 11,033.000 ± 1,290.000	78.6	50.5	50.6
Trichophyton verrucosum, batch 2	2,315.000 ± 127.000	2,277.000 ± 238.000	× × 735.000 ± 97.800	× × 1,380.000 ± 168.000	98.4	31.7	59.6
Trichophyton verrucosum, batch 3	17,500.000 ± 1,475.000	16,700.000 ± 1,590.000	× × 8,100.000 ± 800.000	× × 8,900.000 ± 910.000	95.4	46.3	50.9

after lyophilization. For statistical evaluation of the results Student's t-test was used (Černík 1975).

Results

Viability of 11 dermatophyte strains stored in lyophilized form for 1 year at room and refrigerator temperatures is shown in Table 1. After 1 year storage in refrigerator in 10 of 13 samples more than 78 % elements survived as against 100 % found immediately after lyophilization. This decrease in viability was not statistically significant. However, in the remaining 3 strains, (*M. canis* No. 4043, *Trichophyton ajelloi* and *T. equinum*), stored in refrigerator, significant decreases in viability occurred.

All lyophilized dermatophyte cultures stored at room temperature showed significant to highly significant decreases in viability after 1 year storage. The proportions of surviving fungal elements stored in the dark were 11.4 to 74.6 % depending on the strain, the proportions of those stored in the light were 17.2 to 67.6 % as compared to the initial values determined immediately after lyophilization.

Discussion

Among dermatophytes only strains forming numerous microconidia are suitable for lyophilization and storage as indicated by the above-mentioned writers. This observation has been used in practice for preparation of live lyophilized vaccines against dermatomycoses in animals.

The basis of the Soviet vaccines against cattle trichophytosis (LTF-130), horse trichophytosis (S-P-1), fur-animal and rabbit trichophytosis (Mentavak), and of the Czechoslovak vaccine against cattle trichophytosis are live lyophilized microconidia of the respective manufacturer strains. Long-time storage of large amounts of vials containing the lyophilized vaccine at refrigerator temperature often causes difficulties for the manufacturer. Therefore we attempted to solve the problem by storing lyophilized Czechoslovak vaccination strain of *T. verrucosum* and other dermatophyte strains at room temperature. Our results have shown, however, a significant decrease in viability of lyophilized *T. verrucosum* microconidia after 1 year storage (i.e. shelf life of the vaccine) whereas at refrigerator temperature no substantial changes occurred. At the same time in samples stored in the light slightly higher survival rates were obtained than in those stored in the dark. These results are somewhat surprising as an inhibitory effect of light on viability of dermatophyte cultures is well-known (Buchníček 1974). The explanation lies possibly in greater resistance of lyophilized spores, UV-radiopacity of the vial wall or exposure to light of only superficial layers of lyophilizates. However, the real contribution of these and/or other factors needs further experimental evidence.

Our results obtained with lyophilized 10 dermatophyte strains were similar to those obtained with the vaccination *T. verrucosum* strain. Viability of lyophilized cultures decreased only slightly in refrigerated samples (except for 3 strains) whereas storage at room temperature resulted in significantly to highly significantly decreased viability of all strains. Our data thus quantitatively confirm the results of Rhoades (1970) obtained in stored lyophilized cultures of bacteria, molds and yeast but they are at variance with the data of Sarkisov et al. (1976) who reported that viability of lyophilized microconidia of the Soviet vaccination strain *T. verrucosum* was unchanged or only slightly decreased after one year storage at refrigerator but also at room temperature. Despite these findings the authors recommended a storage

temperature of 4 to 8 °C for the LTF-130 vaccine prepared from microconidia of the above-mentioned strain.

In conclusion, for long-time storage of lyophilized dermatophyte cultured refrigerator temperature is suitable. Although it is possible to recover fungus cultures from lyophilized samples stored at room temperature both in the dark and light for one year, the viability of the organisms is considerably decreased when compared with refrigerated samples.

Udržování kultur dermatofytů v lyofilizovaném stavu

Snížení životaschopnosti houbových elementů u 10 ze 13 zkoumaných lyofilizovaných kultur dermatofytů, udržovaných po dobu 1 roku při teplotě +2 až +6 °C, nebylo statisticky významné. Pouze u 3 lyofilizovaných kmenů, uchovávaných v chladničce, přežívalo po jednom roce méně než 78 % houbových jednotek při porovnání s výchozím množstvím, zjištěným ihned po lyofilizaci. Pokles viability v těchto případech byl statisticky signifikantní. Životaschopnost všech lyofilizovaných kultur po jednoročním skladování při pokojové teplotě byla průkazně až vysoce průkazně snížena. Přitom bylo dosaženo podobných výsledků u vzorků uchovávaných v temnu i na světle.

Содержание культур дерматофитов в лиофилизированном состоянии

Положение жизнеспособности грибных элементов у 10 из 13 исследуемых лиофилизированных культур дерматофитов, содержимых в течение одного года при температуре +2—+6 °C не отличалось статистической значимостью. Лишь у 3 лиофилизированных штаммов, хранящихся в холодильнике, переживало спустя один год меньше 78 % грибных элементов по сравнению с исходным количеством, установленным сразу же после взгонки на холоду. Понижение жизнеспособности в данном случае были статистически значимым. Жизнеспособность всех лиофилизированных культур после годичного хранения при комнатной температуре явно, даже существенно понижалась. При этом было достигнуто сравнимых результатов у образчиков, хранимых в темноте и на свете.

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