

LYOPHILIZATION OF TRICHOPHYTON VERRUCOSUM ORGANISMS

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Abstract

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The effect was studied of lyophilization on the viability of *Trichophyton verrucosum* microorganisms and the percentage of their survival in a lyophilized condition after long-term storage. The amount of surviving *T. verrucosum* elements was found to range from 27 % to 87 % (on average 54 %) as compared with the initial amount before lyophilization. After six months of storage in darkness at 4 °C nearly 100 % of the microorganisms were found to survive, after 12 months 90.5 to 98.5 %, and after 2–3 years approximately 80 % of the *T. verrucosum* microorganisms. The rate of decrease of the viability of lyophilized *T. verrucosum* elements is probably associated with the residual humidity of the lyophilized substance.

Lyophilized substance, viability, residual humidity.

From numerous literary data it is evident that the main originator of cattle trichophytosis in Czechoslovakia and abroad is the dermatophytic fungus *Trichophyton verrucosum* Bodin, 1902 (Čuturič 1968; Refai et al. 1976; Dobšínský et al. 1976; Sarkisov 1976; Pavlas et al. 1979). The resistance of the parasitic form of this species against disinfection means and against unfavourable life conditions is well known (Tkáčik 1969; Ladzianska and Halaša 1975). In spite of this fact it is very difficult to maintain this fungus under laboratory conditions. When maintaining the *T. verrucosum* culture in vitro for a longer length of time unconstant modification and hereditary mutations are formed. From this point of view the method of lyophilization seems to be more suited for long-term preservation of *T. verrucosum* strains. In the literature available data about the effect of lyophilization on the viability of *T. verrucosum* microorganisms are given only in the studies of the Polish author Wawrzkievicz (1976). When using 50 % normal inactivated calf serum as a protective lyophilic medium the above mentioned author was successful in maintaining the *T. verrucosum* lyophilized substance in a living and unchanged condition for a period of one year. However, lyophilization caused a more than 100-fold decrease of the number of living microorganisms as compared with the initial amount, and this represents considerable losses. The aim of the present study was to determine the number of surviving *T. verrucosum* organisms after lyophilization and after a three-year storage using the author's own method of lyophilization.

Materials and Methods

The *Trichophyton verrucosum* Bodin (1902) culture was cultivated in darkness at a temperature of 28 °C on malt-agar. After 14–16 days of growth it was homogenized in saline. Into a thus prepared homogenized substance the protective lyophilic medium (5 % of gelatine and 7.5 % of saccharose) was added in the same amount as the saline. A sample was taken from the suspension obtained and the living organisms of *T. verrucosum* were counted using the plate dilution method; the numbers of microconidia were assessed microscopically. After taking the sample the suspension was divided in the amount determined into penicillin vials and frozen to –50 °C. Drying was performed in a KS-30 lyophilization apparatus for 24 to 48 hours. Vials with the dried substrate were closed with rubber caps in a vacuum. In the lyophilized substance obtained the residual humidity was determined and the numbers of living fungi were counted using the plate method.

When determining the number of living *T. verrucosum* microorganisms using the plate method

the lyophilized substance was diluted with saline in the same ratio as the homogenized sample before lyophilization. In both cases the samples were gradually diluted to a concentration of 10^{-5} and 10^{-6} . From each of these dilutions 0.5 cm^3 was transferred onto Petri dishes with malt-agar and after 7 days of incubation at 28°C in darkness the *T. verrucosum* colonies grown were counted. From the results obtained the number of living *T. verrucosum* organisms in 1 cm^3 of diluted sample was counted.

The number of *T. verrucosum* microconidia in the sample before lyophilization was determined microscopically in a Bürker's chamber.

The residual humidity of the lyophilized substance was assessed from the difference in its weight after lyophilization and after drying at 105°C in a drier for a period of one hour.

Results

A survey of the effect of lyophilization on the viability of *T. verrucosum* micro-organisms is given in Tab. 1. This table also gives the results of microscopical determination of the number of microconidia in the majority of samples before lyophilization. The amount of *T. verrucosum* elements surviving the process of

Table 1
Effect of lyophilization on viability of *Trichophyton verrucosum* organisms

Sample No.	No. of microconidia in 1 cm^3 of sample before lyophilization	No. of viable <i>T. verrucosum</i> in 1 cm^3 of sample		Per cent of surviving <i>T. verrucosum</i> after lyophilization
		before lyophilization	after lyophilization	
1		23,300,000	17,600,000	75.5
2		46,570,000	28,300,000	60.8
3	1,296,000	2,100,000	1,180,000	56.2
4	35,600,000	35,000,000	22,825,000	65.2
5	24,320,000	25,575,000	18,150,000	71.0
6		11,067,000	7,350,000	66.4
7	29,700,000	38,700,000	21,525,000	55.6
8	35,100,000	28,975,000	24,450,000	84.4
9	23,625,000	32,950,000	20,275,000	61.5
10	17,600,000	16,325,000	10,540,000	64.6
11	21,600,000	26,533,000	13,280,000	50.1
12		13,180,000	10,425,000	79.1
13	12,210,000	14,325,000	8,450,000	59.0
14	11,070,000	10,075,000	4,200,000	41.7
15	4,880,000	6,175,000	3,475,000	56.3
16	5,490,000	7,800,000	3,830,000	49.1
17	2,950,000	4,530,000	1,925,000	42.5
18	6,480,000	9,970,000	4,325,000	43.4
19	12,560,000	11,900,000	6,930,000	58.2
20	10,000,000	13,800,000	7,000,000	50.7
21	7,720,000	9,150,000	3,800,000	41.5
22	12,190,000	12,305,000	7,000,000	56.9
23	9,760,000	11,640,000	5,830,000	50.1
24	13,155,000	25,750,000	15,530,000	60.3
25		26,050,000	14,330,000	55.0
26		18,300,000	16,000,000	87.4
27	11,895,000	32,225,000	10,330,000	32.1
28		18,800,000	9,530,000	50.7
29		14,700,000	7,030,000	47.8
30	20,500,000	32,570,000	14,350,000	44.1
31	26,200,000	36,370,000	11,200,000	30.8
32	20,140,000	20,000,000	11,600,000	58.0
33	18,215,000	22,000,000	15,600,000	70.9
34	15,590,000	18,800,000	11,900,000	63.3
35	18,780,000	17,360,000	11,400,000	65.7
36	42,900,000	53,000,000	18,900,000	35.7
37	30,970,000	30,000,000	8,200,000	27.3
38	10,400,000	18,835,000	5,330,000	28.3
39	14,350,000	26,370,000	8,900,000	33.8
Mean values	17,008,000	21,104,000	11,354,000	53.8

Table 2

Survival of *T. verrucosum* in lyophilized form kept in the dark at 4 °C for 6, 12, 18, 24 and 36 months

Sample No.	No. of viable <i>T. verrucosum</i> in 1 cm ³ of sample before lyophilization	Lyophilizate moisture %	Storage of lyophilizate (months)	No. of viable <i>T. verrucosum</i> in 1 cm ³ of sample after the storage time	Per cent of surviving <i>T. verrucosum</i> organisms
40	5,330,000	2.04	6	5,300,000	99.4
41	12,400,000	1.44	6	12,230,000	98.6
42	5,800,000	1.29	12	5,250,000	90.5
43	10,425,000	1.99	12	9,900,000	95.0
44	3,830,000	2.45	12	3,680,000	96.1
45	2,740,000	2.55	12	2,700,000	98.5
46	2,625,000	1.93	18	2,400,000	91.4
47	17,900,000	3.02	18	12,670,000	70.8
48	11,150,000	3.44	18	8,675,000	77.8
49	7,700,000	2.80	18	6,770,000	87.9
50	7,000,000	3.10	24	5,350,000	76.4
51	11,600,000	3.42	24	8,500,000	73.3
52	15,600,000	2.46	24	12,700,000	81.4
53	5,600,000	2.54	36	4,600,000	82.1
54	2,200,000	3.12	36	1,725,000	78.4

lyophilization in the individual samples ranged between 27.3 % and 87.4 % (54 % on average) of the initial number before lyophilization.

Tab. 2 shows the results of the survival of *T. verrucosum* microorganisms maintained for a long period in a lyophilized condition in darkness at 4 °C. After 6 months of storage of the lyophilized substances the viability of *T. verrucosum* remained virtually unchanged. After one year of maintaining the microorganisms in a lyophilized condition 90.5–98.5 % of the initial number before lyophilization survived. When storing for a period of more than one year the number of living *T. verrucosum* elements in the lyophilized substance further decreased and after three years the number of surviving organisms ranged around 80 % of the initial number. The amount of surviving *T. verrucosum* microorganisms was relatively lower in samples with a high residual humidity of the lyophilized substance.

Discussion

Acquiring successful results in the lyophilization of microbiological cultures depends not only on the method of performing the individual stages of lyophilization but also on the quality of the initial biological material. From results obtained by Wawrzekiewicz (1976) it is evident that during lyophilization of *T. verrucosum* culture composed almost exclusively of mycelia the great majority of the organisms dies and the number of surviving elements is about 100 times lower as compared with the initial amount. In the present experiment the *T. verrucosum* culture to be lyophilized was composed of a great number of microconidia (see Tab. 1), a smaller part was formed by mycelium, and sporadically also chlamydo-spores and macroconidia occurred. From the results of the decrease of the number of living organisms after lyophilization it is evident that under the effect of lyophilization probably not only the mycelium was damaged but also a certain part of the microconidia. In spite of this fact the amount of surviving *T. verrucosum* microorganisms was relatively high and ranged from 27 % to 87 % (54 %

on average). These values outnumber the results given during the lyophilization of bacteria when the viability decreased mostly to 5–25 % (Sturdza et al. 1968).

In the present experiments the lyophilized substances were stored at a temperature of 4 °C in darkness because the inhibitory effect of visible light on dermatophytes is well known (Buchniček 1974). The viability of *T. verrucosum* maintained under the conditions given decreased in the course of one year only insignificantly (by 1.5 to 9.5 %). After a longer period of maintaining the lyophilized substances the number of surviving microorganisms ranged from 70–80 % up to three years of storage (the period of investigations). Besides, in samples with a higher residual humidity of the lyophilized substance the decrease of the viability of *T. verrucosum* was relatively higher.

A direct dependence between the residual humidity of lyophilized microorganisms and the percentage of their lethal damage is known from data in bacteria (Proom 1951; Sturdza et al. 1968). However, in the same way as a too high amount of residual humidity the viability of bacteria can be unfavourably affected also by excessive drying (Hutton et al. 1951). In lyophilized *T. verrucosum* substances with a high residual humidity in our case the germination of microconidia probably occurred and thus also a decrease of their viability during long-term storage. In the present study it was not possible to determine the precise range of residual humidity of the lyophilized substance under which the viability of the preserved *T. verrucosum* microorganisms was the highest and this problem will have to be further investigated in greater detail.

Lyofilizace mikroorganismů *Trichophyton verrucosum*

Byl sledován vliv procesu lyofilizace na životaschopnost mikroorganismů *Trichophyton verrucosum* a procento jejich přežívání v lyofilizovaném stavu při dlouhodobém skladování. Bylo zjištěno, že množství přežívajících elementů *T. verrucosum* po lyofilizaci se pohybuje od 27 % do 87 % (průměrně 54 %) v porovnání s výchozím počtem před lyofilizací. Po 6 měsících skladování v temnu při 4 °C přežívalo téměř 100 % mikroorganismů, po 12 měsících 90,5 až 98,5% a po 2 až 3 letech kolem 80 % mikroorganismů *T. verrucosum*. Velikost snížení životnosti lyofilizovaných zárodků *T. verrucosum* pravděpodobně souvisí se zbytkovou vlhkostí lyofilizátu.

Лиофилизация микроорганизмов *Trichophyton verrucosum*

Проводились исследования влияния процесса лиофилизации на жизнеспособность микроорганизмов *Trichophyton verrucosum* и процента их переживания в лиофилизированном состоянии при длительном хранении. Было установлено, что количество переживающих элементов *T. verrucosum* после лиофилизации достигает пределов 27 %–87 % (в среднем 54 %) по сравнению с исходным количеством перед лиофилизацией. После 6 месяцев хранения в темном помещении при температуре 4 °C переживало почти 100 % микроорганизмов, после 12 месяцев 90,5–98,5 % и после 2–3 лет приблизительно 80 % микроорганизмов *T. verrucosum*. Понижение жизнеспособности лиофилизированных элементов *T. verrucosum* вероятно связано с остаточной влажностью лиофилизата.

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