CZECH MYCOL. 55 (3–4), 2003

Survival rate of Trichophyton equinum and T. verrucosum mutants at lyophilisation

ALOIS RYBNIKÁŘ[1], MILAN HEJTMÁNEK[2] and EVŽEN WEIGL[2]

[1] Bioveta a. s., 683 23 Ivanovice na Hané, Czech Republic
[2] Medical Faculty of the Palacký University, 775 15 Olomouc, Czech Republic

Rybnikář A., Hejtmánek M. and Weigl E. (2003): Survival rate of Trichophyton equinum and T. verrucosum mutants at lyophilisation. – Czech Mycol. 55: 273–276

Trichophyton equinum and *T. verrucosum* mutants were prepared from monoconidial wild-type strains by induction with ultraviolet radiation. The percentage of elements surviving at lyophilisation was approximately the same as or higher than that of relative wild-type strain with four of twelve *T. equinum* mutants and five of nine *T. verrucosum* mutants. With remaining eight *T. equinum* mutants and four *T. verrucosum* mutants the ability to survive at lyophilisation was lower in comparison with the wild-type strains.

Key words: lyophilisation, dermatophytes, mutants

Rybnikář A., Hejtmánek M. a Weigl E. (2003): Přežívání mutantů Trichophyton equinum a T. verrucosum při lyofilizaci. – Czech Mycol. 55: 273–276

Mutanti *Trichophyton equinum* a *T. verrucosum* byli připraveni z monokonidiálních divokých kmenů indukcí UV-zářením. U čtyř z dvanácti mutantů *T. equinum* a u pěti z devíti mutantů *T. verrucosum* bylo procento elementů přežívajících při lyofilizaci přibližně stejné nebo i vyšší než u příslušného divokého kmene. U zbývajících osmi mutantů *T. equinum* a čtyř mutantů *T. verrucosum* byla schopnost přežívat lyofilizaci oproti výchozím kmenům nižší.

INTRODUCTION

First studies of preparation of dermatophyte mutants by means of ultraviolet radiation were published long ago by Emmons and Hollaender (1939, 1945). Their studies were above all aimed at taxonomy. Growth, morphological, anatomical and biochemical properties of mutants of dermatophyte fungi induced by ultraviolet radiation were also studied by other authors (Lenhart 1965, 1969; Hejtmánek et al. 1986; Hejtmánek and Geschwinderová 1988). We were interested to know to what extent induced mutation affects the ability of dermatophytes to survive at lyophilisation. We selected 12 *Trichophyton equinum* mutants and 9 *T. verrucosum* mutants prepared by means of ultraviolet radiation to solve these problems. The formation of microconidia in all tested mutants reached the level of wild-type strains.

MATERIALS AND METHODS

Wild-type strains of *Trichophyton equinum* no. 4043 and *T. verrucosum* no. 650 as well as their mutants induced by ultraviolet radiation (Hejtmánek et al. 1986, Weigl and Hejtmánek 1988) were cultivated on malt agar. Some of tested strains (wild-type 650, M-9, M-31, M-141) have been deposited in Czech Collection of Microorganisms Brno, the other strains have been deposited in the collection of microorganisms in Bioveta Ivanovice na Hané company. Cultivation took place in the dark at a temperature of 28 °C for a period of 12–16 days. Grown cultures were homogenised in a physiological saline environment until a homogeneous suspension was formed. As a protective lyophilisation medium a water solution of 5 % gelatine and 7.5 % saccharose was added in the same amount as the saline solution. The suspension formed was dispensed standardly into glass medicine bottles under stable agitation and lyophilised (Rybnikář et al. 1983). The medicine bottles with lyophilised strains were closed with rubber airtight stoppers under vacuum.

The number of viable CFU (colony forming units) was established before lyophilisation and within 5 days after lyophilisation terminated with all strains being investigated. Inoculation of standardly diluted samples on Sabouraud's agar were performed by the plate dilution method (Rybnikář 1981).

RESULTS

If the a number of CFU before lyophilisation is set to 100 %, then 85 % of CFU survive lyophilisation with the wild-type strain of *Trichophyton equinum*. The same or a higher percentage of survival rate at lyophilisation was found at four of twelve *T. equinum* mutants. The number of viable elements after lyophilisation amounted to 32.4–70.9 % (Table 1) with the remaining eight *T. equinum* mutants.

With the wild-type strain of *T. verrucosum* the number of CFU fell to 58.9 % after lyophilisation in comparison with the state before lyophilisation (Table 2).

With five *T. verrucosum* mutants of nine being investigated the fall of relative viability was practically the same or even distinctly lower at lyophilisation. The viability of four *T. verrucosum* mutants was relatively low in comparison with wild-type strain (27.3–49.8 %) after lyophilisation.

DISCUSSION

Lyophilisation presents one of the most utilized methods of long-term preservation of microscopical fungi (Bunse and Steigleder 1991). A fact of common knowledge is that only spore-bearing strains of micromycetes are suitable for this method of preservation. The best results were obtained with cultures forming a great number

Table 1. Survival rate of *Trichophyton equinum* strains at lyophilisation.

Strain number*	CFU number/ml of standard suspension		CFU % surviving at lyophilisation
	before lyophilisation	after lyophilisation	
Wild-type 4043	8,510,000	7,230,000	85.0
M-3	4,125,000	3,550,000	86.1
M-5	4,090,000	2,900,000	70.9
M-48	7,900,000	4,975,000	63.0
M-70	3,775,000	2,425,000	64.2
M-77	5,150,000	4,350,000	84.5
M-85	7,950,000	3,800,000	47.8
M-88	1,064,000	350,000	32.9
M-92	6,975,000	4,125,000	59.1
M-94	3,122,000	1,010,000	32.4
M-141	8,650,000	8,450,000	97.7
M-146	1,851,000	750,000	40.5
M-159	8,700,000	7,900,000	90.8

* Strains M-3 to M-159 are mutants prepared from wild-type no. 4043 by means of ultraviolet radiation.

Table 2. Survival rate of *Trichophyton verrucosum* strains at lyophilisation.

Strain number*	CFU number/ml of standard suspension		CFU % surviving at lyophilisation
	before lyophilisation	after lyophilisation	
Wild-type 650	8,400,000	4,950,000	58.9
M-1	3,175,000	2,410,000	75,9
M-8	4,925,000	3,175,000	64.5
M-9	8,825,000	6,770,000	76.7
M-25	1,685,000	460,000	27.3
M-26	3,250,000	2,338,000	71.9
M-31	10,850,000	6,800,000	62.7
M-39	1,920,000	675,000	35.2
M-41	3,424,000	1,150,000	33.6
M-62	6,150,000	3,063,000	49.8

* Strains M-1 to M-62 are mutants prepared from wild-type no. 650 by means of ultraviolet radiation.

of microconidia (Rybnikář et al. 1983, Rybnikář 1994) at lyophilisation of dermatophytes. Therefore, we selected for our experiments mutants with which the forming of these spores was not expressively reduced in comparison with initial strains.

It is obvious from former studies (Hejtmánek et al. 1986, Hejtmánek and Geschwinderová 1988, Weigl and Hejtmánek 1988) that some biological properties of dermatophyte mutants prepared by means of ultraviolet radiation are in comparison with wild-type strains markedly different. Avirulent mutants or mutants hav-

ing reduced virulence have originated from wild-type, virulent types of *T. equinum* and *T. verrucosum*. Above all their growth rate, temperature sensitivity, micro- and macromorphology, keratinolytic activity as well as biochemical properties are different. The results of this study show that the induced mutation of dermatophyte strains can strongly decrease or even slightly increase their sporulation. The ability to survive at lyophilisation was relatively decreased in some mutants in comparison with the wild-type strain. However, with other mutants it was not affected negatively. With several strains prepared by ultraviolet radiation even a higher survival rate at lyophilisation was found in comparison with the wild-type strain. These results were unexpected. However, they showed that it is possible to obtain more advantageous properties of new dermatophyte strains prepared by way of mutation.

The methods of induced mutation and selection of dermatophytes are used not only in theoretical studies, but also in industry. The mutant strains of *T. equinum* and *T. verrucosum* prepared by ultraviolet radiation form the basic effective part of freeze-dried antimycotic vaccines produced at Bioveta Ivanovice na Hané (Rybnikář et al. 1990, Rybnikář et al. 1996).

REFERENCES

BUNSE T. and STEIGLEDER G. K. (1991): The preservation of fungal cultures by lyophilization. – Mycoses 34: 173–176.

EMMONS C. W. and HOLLAENDER A. (1939): The influence of monochromatic ultraviolet radiation on the rate of variant production in Trichophyton mentagrophytes. – Genetics 24: 70–71.

EMMONS C. W. and HOLLAENDER A. (1945): Relation of ultra-violet-induced mutations to speciation in dermatophytes. – Arch. Dermatol. 52: 257–261.

HEJTMÁNEK M. and GESCHWINDEROVÁ J. (1988): Temperature-dependent dimorphism and growth rate of Trichophyton equinum mutants. – Acta Univ. Palacki. Olomuc. (Olomouc), Fac. Med. 120: 23–40.

HEJTMÁNEK M., WEIGL E. and HEJTMÁNKOVÁ N. (1986): Mutants of Trichophyton verrucosum. – Acta Univ. Palacki. Olomuc. (Olomouc), Fac. Med. 114: 149–164.

LENHART K. (1965): Killing and mutagenic effect of UV-radiation on spores of Trichophyton terrestre Durie et Frey 1957. – Z. Allg. Microbiol. 5: 222–227.

LENHART K. (1969): Griseofulvin-resistant mutants in dermatophytes. I. The frequency of spontaneous and UV-induced mutants. – Mykosen 12: 655–660.

RYBNIKÁŘ A. (1981): Lyophilization of Trichophyton verrucosum organisms. – Acta Vet. Brno 50: 73–77.

RYBNIKÁŘ A. (1994): Long-term maintenance of lyophilized fungal cultures of the genera Epidermophyton, Microsporum, Paecilomyces and Trichophyton. – Mycoses 39: 145–147.

RYBNIKÁŘ A., DITRICH O. and PYTELA F. (1983): Lyophilization of some cultures of dermatophytes. – Čes. Mykol. 37: 93–98. (In Czech with English summary).

RYBNIKÁŘ A., CHUMELA J. and VRZAL V. (1990): Development of the antimycotic vaccines in Bioveta, Ivanovice na Hané. – Veterinářství 40: 350. (In Czech).

RYBNIKÁŘ A., VRZAL V., CHUMELA J., HEJTMÁNEK M. and WEIGL E. (1996): Vaccination of cattle against trichophytosis using the Czech vaccines. – J. Mycol. Med. 6: 93–94.

WEIGL E. and HEJTMÁNEK M. (1988): Mutants of Trichophyton equinum. – Acta. Univ. Palacki. Olomuc. (Olomouc), Fac. Med. 119: 123–136.