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Use of nystatin for random spore selection in the yeast *Saccharomycopsis lipolytica*

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Nystatin was used to develop a new method to select spores of the yeast *Saccharomycopsis lipolytica*. At low concentrations nystatin killed preferently growing cells of this yeast. At high concentrations nongrowing cells were affected as well. In contrast, spores were not sensitive to nystatin action. This differential response to the antibiotic suggested its use to select spores from sporulated yeast cultures.

Separation of spores from vegetative cells is an important prerequisite for many methods of genetic analysis in yeast. Therefore, several procedures have been developed to isolate spores from *Saccharomyces* (*Sacch.*) *cerevisiae* (reviews s. SHERMAN 1975, MORTIMER and HAWTHORHE 1975). Spores of the yeast *Saccharomycopsis* (*S.*) *lipolytica* have been separated by the paraffin oil method (EMEIS and GUTZ 1958, GAILLARDIN *et al.* 1973) and canavanine resistance method (SHERMAN and ROMAN 1963, BASSEL *et al.* 1971). Besides these methods both ether vapour (DAWES and HARDIE 1974) and acetone vapour (EGEL 1977) have been used for the isolation of spores from *S. lipolytica* (BARTH 1980).

In this paper we describe a simple method which makes use of nystatin to select spores from *S. lipolytica*.

Strains and materials: *S. lipolytica* strains used throughout the experiments have been described previously (BARTH and WEBER 1983, 1984). Nystatin was obtained from the Department of Antibiotic Chemistry of the Central Institute for Microbiology and Experimental Therapy, Jena.

Media: Minimal medium (MMT) and complete medium (YEP-G) are described previously (BARTH and KÜNKEL 1979). The composition of the sporulation medium (CSM) will be published elsewhere (BARTH and WEBER 1984).

Methods: Nystatin was suspended in absolute ethanol at concentrations of 1 mg or 2 mg per ml. The suspension was always freshly prepared and immediately applied to the culture. Ethanol concentrations up to three percent did not significantly affect the growth of cells in glucose containing medium.

Sporulation: The method used to induce sporulation will be described elsewhere (BARTH and WEBER, in preparation).

Random spore selection: Sporulated strains were suspended in YEP-G and shaken for 2 hours to activate vegetative cells. This time was to short to induce germination of spores. The pH was then adjusted to 4.5 to 5.0 by the addition of HCl and nystatin solution was added to the desired concentration. These cultures were shaken another 1.5 hours, centrifuged, and washed twice with water. Subsequently cells were resuspended in helicase solution (200 mg/7 ml aqua dest.) and incubated for one more hour. The cells were then sedimented again, washed twice with water and resuspended in aqua dest. After sonication (BRANSON sonicator, 5 × 1 min) suspensions were spreaded on YEP-G plates and incubated at 28 °C. Two days later YEP-G plates were replica-plated onto appropriate minimal medium plates and CSM plates. Colonies of diploid cells were coloured brownish after sporulation in contrast to the white unsporulated colonies.

The frequency of survival after nystatin treatment was estimated for several haploid and diploid strains of *S. lipolytica*. Nystatin strongly inactivates growing haploid

Table 1
Inactivation of *S. lipolytica* cells by nystatin (10 µg/ml) after cultivation in nonsupplemented MMT + 1% glucose for 6 hours

a) Inactivation of growing cells of haploid (H 194, H 195, H 222) and diploid strains (B1, B215)		b) Inactivation of nongrowing cells of auxotrophic mutant strains	
Strains	Frequency of survival	Strains	Frequency of survival
H 194	5×10^{-4}	H 194-15	5×10^{-1}
H 195	8×10^{-4}	H 195-5	3×10^{-1}
H 222	6×10^{-4}	H 222-20	3×10^{-1}
B 1	8×10^{-4}		
B 215	9×10^{-4}		

Table 2
Inactivation of nongrowing cells of *S. lipolytica* by different concentrations of nystatin. Nongrowing cells from stationary growth phase were used

	Survival of cells after treatment with different concentration of nystatin		
	10 µg/ml	20 µg/ml	40 µg/ml
H 195-5	3×10^{-1}	2×10^{-2}	6×10^{-3}
B 157	6×10^{-1}	3×10^{-2}	8×10^{-3}
B 215	5×10^{-1}	1×10^{-2}	3×10^{-3}

and diploid cells of *S. lipolytica* (Table 1a) but showed little effect on nongrowing cells like auxotrophic mutants when incubated in nonsupplemented minimal medium (Table 1b). Therefore, nystatin was useful for enrichment of *S. lipolytica* mutants by special procedures (GAILLARDIN *et al.* 1973, BARTH 1980, BARTH and WEBER 1983). Nystatin inactivates nongrowing haploid and diploid cells of *S. lipolytica* stronger at higher concentrations than at lower concentrations (Table 2). These data suggested the use of nystatin to kill selectively vegetative cells in sporulated cultures in order to select ascospores of this yeast. Spores were expected not to be killed since they were protected by the ascus wall and their own thick spore wall as well as the material covering spores (WEBER 1979, BARTH and WEBER, in preparation). Therefore, the procedure for random spore selection described in the methods and materials sections was elaborated. This method has been applied to several sporulated diploid cultures. The portion of diploid cells among the survivers was easily detectable by brownish colour of sporulated colonies on CSM. In all cases tested so far no or only one to two diploid cells were observed among five thousand surviving cells per strain investigated. No discriminating effects by nystatin were observed among spore isolates using several auxotrophic markers or mating type alleles A and B (Table 3).

Our data show that nystatin may successfully be used for spore selection and for random spore analysis in *S. lipolytica*. SAMSONOVA and BÖTTCHER (1980) used nystatin to select genetic segregants in *Pichia guilliermondii* strains. The authors discussed the possibilities that nystatin either induced haploidization or, what seemed more likely to us, that it selectively killed vegetative cells thus leading to the selection of spores in this yeast, too.

Table 3
Segregation pattern among survivied cells after nystatin treatment of two sporulated diploid strains
a) Segregation pattern of auxotrophic markers

Diploid strain	Genotype	Random spore type	
		P	NP
B 108	ade, LYS+/ADE+, lys	1150	1174

$\chi^2 = 0.25$ $0.80 > P > 0.50$

b) Segregation pattern of mating type alleles

Diploid strain	Genotype	Mating type of random spores	
		A	B
B 86	A/B	71	67

$\chi^2 = 0.12$ $0.80 > P > 0.50$

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