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GENERAL BIOLOGY

The Phenomenon of Spore Killing in *Schizosaccharomyces pombe* Hybrids

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Both genetic and molecular properties of the fission yeast *Schizosaccharomyces pombe* have been characterized in the literature in almost as much detail as these properties of the classical yeast object *Sacharomyces cerevisiae* [1]. It is presently beyond doubt that these yeast species are separated by a very large molecular "hiatus," which corresponds to the difference between orders rather than genera or families [2]. Unfortunately, the genetic pool of *Sch. pombe* studied thus far is very limited and restricted mainly to laboratory genetic strains [3]. In contrast to *S. cerevisiae* [4], natural polymorphism of *Sch. pombe* remains almost unknown. The literature contains only two reports on natural isolates of *Sch. pombe,* in which transition from homothallism to heterothallism [5] and molecular polymorphism of the mating type locus [6] were observed. Single natural isolates of *Sch. pombe* were subjected to taxonomic research [7–9]. It should be noted that the genus *Schizosaccharomyces* is a small but very heterogeneous group of microorganisms [10].

Comparative hybridization analysis of the *Sch. pombe* strains of different geographical origin revealed the phenomenon of spore killing in interstrain crossing. The process of intraspecies hybrid degeneration of yeast spores has been described for the first time in our works.

The objects of the study were nine strains of *Sch. pombe* obtained from Centraalbureau voor Scimmelcultures, Utrecht, The Netherlands (CBS): CBS 356 (Africa), CBS 357 (Jamaica), CBS 1043 (Taiwan), CBS 1057 (Sweden), CBS 1061 (Tahiti?), CBS 2628 (Pakistan), CBS 5557 (Spain), CBS 5680 (Poland), and CBS 5682 (South Africa). The places of isolation of the strains are given in parentheses. Detailed description of these strains can be found in the Internet: http://www.cbs.knaw.nl. The tester strains 972 h^- (CBS 7264) and 975 h^+ (CBS 7265) used in the study were from the laboratory of H. Heslot (INRA, Paris).

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Yeast cells were grown at 28°C on the YEA complete medium containing 5 g/l yeast extract, 30 g/l glucose, and 20 g/l agar. The MMA minimal selective medium contained 10 g/l glucose; solution of salts, vitamins, and microelements; and 20 g/l agar [11]. Crossing was performed in the SPA medium containing 10 g/l glucose, 1 g/l KH_2PO_4, vitamin complex, and 30 g/l agar [11]. The MEA medium containing 5 g/l wort extract, 30 g/l glucose, and 20 g/l agar was used for yeast growing and inducing spore formation at 25°C. Ascospores were isolated using a micromanipulator needle. Ascus walls were destroyed using the garden snail (*Helix pomatia*) stomach juice (SJ). Random spore analysis was conducted using the method of Leupold [11]. Sporulating cultures were treated with SJ for 6 h at 28°C and with 30% ethanol for 30 min at 18°C. In some cases, the duration of treatment with SJ and 30% ethanol was reduced to 3 h and 10 min, respectively.

Homothallism was detected by changes in the color of colonies grown on MEA medium in the presence of iodine [11]. Under these conditions, spores of *Schizosaccharomyces* yeast, containing starch-like compounds in cell wall, are stained black. Strains CBS 356, CBS 1043, CBS 5557, CBS 5680, and CBS 5682 were determined as homothallic, and strains CBS 357, CBS 1057, CBS 1061, and CBS 2628, as heterothallic.

The type of mating of heterothallic strains was determined by crossing with the tester strains 972 h^- and 975 h^+ on the SPA medium and subsequent replica-plating with velvet onto MEA medium. Crossing of the tester strain 975 h^+ with either of two heterothallic strains (CBS 357 and CBS 2628) resulted in positive iodine reaction. Neither of the tester strains was capable of crossing with strains CBS 1057 or CBS 1061.

Monosporic clones were obtained in sporulating homothallic cultures, whereas heterothallic cultures were used as monocell cultures. The following monosporic clones were selected as clones with a high viability of ascospores: 356-4B (75%), 1043-1B (100%), 5557-1A (100%), 5680-2B (76%), and 5682-2C (100%). Both monosporic and monocell clones were marked with auxotrophic mutations induced by ultraviolet radiation or nitrosoguanidine. Only *ura*- and *ade*-

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Table 1. Genetic analysis of intrastrain hybrids of *Sch. pombe*

No.	Origin of hybrids, genotypes	Number of spores isolated with a micromanipulator	Spore viability, %	Segregation of control markers in random spore analysis*			
				AB	aB	Ab	ab
1	1043 × 1043						
	ade1/*ura6*	69	53	9	5	6	7
2	5680 × 5680						
	ade1/*ura*5	55	64	11	9	5	10
3	5557 × 5557						
	ade2/*ura4*	117	63	24	17	15	15
4	356 × 356						
	ade7/*ura16*	128	72	22	20	21	20
5	357 × 357						
	ade1/*ura2*	99	57	12	17	17	14
6	5682 × 5682						
	ade14/*ura22*	92	71	16	15	17	16

* a and b are the auxotrophies of the first (before the sign of crossing) and the second parent, respectively; A and B are the prototrophies of the same parents.

mutants were induced in all strains. Homothallic auxotrophic mutants were found to have low sporulation activity. The viability of spores was tested only in strains 1043 *ura6* and 1043 *ura7*. The spore viability of these strains was 81 and 91%, respectively.

Markered strains were crossed with one another in different combinations by means of combined inoculation of one-day-old cultures on SPA medium. The resulting yeast cultures were spread on a selective minimal medium 14–16 h after the combined inoculation. Two days later, the hybrid diploid colonies were cloned on a minimal medium, and after two days, more streaks of these clones were plated on the MEA medium for subsequent growth and sporulation. The stability of diploid hybrids was monitored microscopically at all stages of replating. Hybrids with premature sporulation were not taken into account. Hybrids of strain CBS 1043 were abundant but had a very short diplophase. The duration of these crosses on SPA medium did not exceed 2 h. Immediately after the crossing, the resulting mixed culture was cloned on the MMA medium. Growing and sporulating colonies were subjected to tetrad analysis without further passages.

Intrastrain control hybrids nos. 1–6 produced many asci with well-developed spores. The efficiency of spore germination in these hybrids ranged from 53 to 72%. All intrastrain hybrids were characterized by normal meiotic segregation of control markers (Table 1). The efficiency of sporulation of all interstrain hybrids (nos. 7–27) was also sufficiently high. Their asci contained four spores each. Usually, the size and shape of the spores did not go beyond normal limits. However, when isolated with a micromanipulator and inoculated on the complete medium, these ascospores, with rare exception, did not produce colonies (Table 2).

Random spore analysis of interstrain hybrids revealed all classes of segregants, including double auxotrophic recombinants (Table 2). Probably, overestimation of the number of prototrophic clones was due to the fact that some vegetative diploid cells survived after ethanol treatment. The results of our experiments suggest that the strains analyzed are genetically related and belong to the same biological species. This conclusion is also supported by a high level of DNA/DNA reassociation of the type culture CBS 356 with strains CBS 357, CBS 1061, and CBS 5557 (92–98%) [9].

Why were numerous intraspecies hybrids of *Sch. pombe* obtained in our experiments sterile? Our review of the literature showed that a similar phenomenon of spore killing was observed in some mycelial ascomycetes possessing spore killer (Sk) genes.

The killer determinants of *Neurospora* have been studied most thoroughly [12]. Four Sk genes were found in three species of *Neurospora*. In hybrids between killer and sensitive strains, only 50% of ascospores are viable. The spores that have the genotype of the sensitive parent die. Genes Sk are not linked to the mating type and have no effect on the spore viability in the parent strains. In hybrids of different killer strains of *Neurospora* (SK–3k × SK–2k), all spores are nonviable in the majority of asci [13]. The presence of different killer genes Sk in different populations may facilitate genetic isolation of the emerging species.

Spore killers were also found in *Podospora anserina*, *Gibberella fujikuroi* (*Fusarium moniliforme*), and *Cochliobolus heterostrophus* [14, 15]. The effects of the spore killer in yeast are similar to the postmeiotic death of spores or gametes in higher plants and animals.

Table 2. Genetic analysis of interstrain hybrids of *Sch. pombe*

No.	Origin of hybrids, genotypes	Number of spores isolated with a micromanipulator	Spore viability, %	Segregation of control markers in random spore analysis*			
				AB	aB	Ab	ab
7	5680 × 5682						
	ade1/*ura4*	48	0	85	11	51	4
	ura5/*ade16*	101	1	129	49	15	38
8	2628 × 5680						
	ade1/*ura5*	119	0	130	13	18	7
9	5680 × 1043						
	ade1/*ura6*	188	21	18	6	10	5**
	ura5/*ade1*	52	8	57	56	44	43
10	357 × 1057						
	ura2/*ade1*	96	0	122	27	55	52
11	356 × 5557						
	ade3/*ura7*	132	0	65	13	10	16
12	356 × 1043						
	ura16/*ade1*	100	0	198	36	13	77
13	356 × 5680						
	ura16/*ade1*	111	3	94	61	11	7
	ade12/*ura5*	24	0	187	13	12	11
14	356 × 5682						
	ura16/*ade14*	92	0	76	105	17	8
15	5680 × 357						
	ade1/*ura2*	24	0	54	86	58	63
	ura5/*ade1*	100	0	63	69	59	64
16	5680 × 5557						
	ura5/*ade3*	101	3	36	38	27	42
17	1043 × 357						
	ade1/*ura2*	100	0	217	2	17	11
18	1043 × 5557						
	ade1/*ura7*	129	0	58	102	15	87
19	2628 × 5557						
	ade7/*ura7*	92	0	168	6	37	30
20	5557 × 357						
	ura7/*ade1*	116	4	90	114	59	62
	ade3/*ura2*	38	0	53	76	40	66
21	5557 × 1057						
	ura7/*ade1*	57	0	186	0	113	20
22	2628 × 357						
	ade7/*ura2*	88	0	55	14	28	33
23	2628 × 357						
	ade7/*ura6*	100	0	154	31	34	33
24	2628 × 5682						
	ade7/*ura4*	100	0	42	18	138	18
25	5682 × 5557						
	ade14/*ura7*	70	10	97	60	66	3
26	5682 × 1043						
	ura4/*ade1*	60	7	171	27	48	27
27	5682 × 357						
	ura4/*ade1*	51	2	140	45	151	69

* See the note to Table 1.
** Spores were isolated with a micromanipulator.

It should be noted that spore killers have not been found in yeast so far.

The *Sch. pombe* strains studied in this work were isolated from different places geographically remote from one another. Probably, each strain has specific factors providing the killing of hybrid spores but having no effect on the spore viability in the parent strains.

The collection used in this work contained neither strains with identical killer factors nor strains without killer factors. Probably, a larger number of natural isolates of *Sch. pombe* should be examined to study genetic and molecular mechanisms of this phenomenon.

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