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EXPERIMENTAL ARTICLES

Hybrid Sterility of the Yeast *Schizosaccharomyces pombe*: Genetic Genus and Many Species in statu nascendi?

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Abstract—A phenomenon of ascospore death was observed in a number of *Schizosaccharomyces pombe* inter-strain hybrids. Meiotic recombination of the control parental auxotrophic markers was, however, observed in random ascospore analysis. Genetic and molecular biological data indicated existence of at least geographical divergence of the genomes in *Sch. pombe* populations. Classification of the genus, species, and varieties of these yeasts is discussed.

Keywords: *Schizosaccharomyces pombe*, hybridization, phylogenetics, RAPD-PCR, polymorphism of chromosomal DNA

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The fission yeast *Schizosaccharomyces pombe* is of great interest for basic and applied investigations [1–3]. This yeast is close to the classical yeast object *Saccharomyces cerevisiae* in its degree of genetic and molecular study. As long as two decades ago, it became clear that the molecular hiatus between these yeasts was enormous, corresponding to different orders rather than genera or families [4]. Indeed, the yeast *Sch. pombe* occupies a special position in the phylogenetic tree of fungi [5]. It is probably the ancestor form of the present-day ascomycetes. Moreover, an opinion exists that higher eukaryotes (plants and animals) originated from this yeast [6, 7]. Importantly, in many aspects, fission yeasts are more similar to mycelial fungi, plants, and animals than budding yeasts [8] and are therefore the preferable model for studying the cells of higher eukaryotes [9].

The yeast *Sch. pombe* actively ferments sugars and is used to produce alcoholic beverages, predominantly in countries with hot climates, as well as to lower the acidity of grape must and wine [10–12].

In our preliminary communication [13], we described the phenomenon of spore killing in *Sch. pombe* hybrids. Taking into account our additional data [14] on important strains (genetic lines, *Sch. kambucha* nom. nud.) and the newly available evidence [15] of a wide karyotypic diversity of natural *Sch. pombe* strains, we deemed it necessary to publish a special article on the obvious relationship between the phenomenon of ascospore killing in the interstrain hybrids and the chromosomal polymorphism of *Sch. pombe*.

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MATERIALS AND METHODS

Strains and media. Eleven *Sch. pombe* strains of different geographic origin (Table 1), mainly obtained from Centraalbureau voor Schimmelcultues (CBS), Utrecht, The Netherlands, and the genetic lines 972 h^- (CBS 7264) and 975 h^+ (CBS 7265) obtained by us in 1991 from H. Heslot laboratory (INRA, Paris), served as the study subjects. The homothallic strain *Sch. kambucha* (nom. nud.) SPK 571 was kindly provided by A.J.S. Klar (NCI, Frederick, United States) [16, 17]. Full information about the CBS strains used is available online at http://www.cbs.knaw.nl. The origin of the genetic lines should be noted. The heterothallic and haploid strains 972 h^- and 975 h^+ of different mating types [18] were obtained from the culture of CBS 1042 isolated from grape juice in Switzerland [19] and initially identified as *Sch. liquefaciens* Osterwalder. This culture was subsequently re-identified as *Sch. pombe* [20]. It is widely used in genetic studies in a number of laboratories worldwide. The strain CBS 356 originating from the Kral collection in Vienna is the type culture of *Sch. pombe* [21].

The yeast was grown at 28°C on complete YEA medium of the following composition (g/L): yeast extract, 5; glucose, 30; agar, 20. The composition of MMA selective basal medium (g/L) was as follows: glucose, 10; salt, vitamin, and mineral solution, agar, 20 [22].

Table 1. Origin of the *Schizosaccharomyces pombe* strains studied

Strain no. in the collections*		Source and place of isolation	Original name
CBS	VKPM		
356^T	2576	Kral collection, Africa	*Sch. pombe* Lindner 1893
357^T	2587	Cane syrup, Jamaica	*Sch. mellacei* Jörgensen 1909
1043^T	2588	Cane syrup, Taiwan	*Sch. formosensis* Nakazawa 1914
1057	2589	Brewer's yeast, Sweden	*Sch. pombe*
1061^T	2590	Cane syrup, Tahiti	*Sch. taito* Nakazawa 1914
2628	2591	Palm wine, Pakistan	*Sch. pombe*
5557^T	2592	Grapes, Spain	*Sch. malidevorans* Rankine et Fornachon 1964
5680^T	2593	Apples, Poland	*Sch. pombe* var. *acidodevoratus* Dittrich 1964
5682	2594	Bantu beer, SAR	*Sch. pombe*
7264 ($972\ h^-$)	2595	Grape juice, Switzerland	*Sch. liquefaciens* Osterwalder 1924
7265 ($975\ h^+$)	2596	Grape juice, Switzerland	*Sch. liquefaciens* Osterwalder 1924
SPK571	–	Kambucha fungal ferment, China	*Sch. kambucha* Singh et Klar (nom. nud.)

* Abbreviated collection names: VKPM, All-Russian Collection of Industrial Microorganisms, Moscow; VKM, All-Russian Collection of Microorganisms, Moscow. The cultures of CBS 7264 and CBS 7265 originate from the same strain CBS 1042^T = VKM Y-663. Correspondence of the strain numbers from different collections: CBS 356 = VKM Y-658, CBS 357 = VKM Y-652, CBS 1042 = VKM Y-663, CBS 1061 = VKM Y-665. The strain SPK571 is absent in the CBS collection. T, designates the type culture.

Hybridization analysis. Hybridizations were carried out on SPA medium (g/L): glucose, 10; KH_2PO_4, 1; vitamin complex, agar, 30 [22]. MEA medium containing the following (g/L) was used for growth and sporulation at 25°C: malt extract: 5; glucose, 30; agar, 20. Ascospores were isolated with a micromanipulator needle; the ascus membranes were dissolved with the digestive juice of garden snail (*Helix pomatia*) (SDJ). A random spore sample was obtained according to the method of Leupold [22]. The sporulating cultures were treated with SDJ for 6 h at 28°C and with 30% ethanol for 30 min at 18°C. In some cases, the treatment time was decreased: SDJ, 3 h and 30% ethanol, 10 min.

Monospore or monocellular haploid clones were marked by auxotrophic mutations induced by ultraviolet irradiation or nitrosoguanidine. The strains with complementary auxotrophic mutations were hybridized on SPA at 25°C. A mixture of 24-h cultures was placed on the agar surface; after 6–14 h, the yeast was transferred to MMA, two drops of 0.9% NaCl were added, and the mixture was lawn-inoculated. The reversion to prototrophy was controlled by plating the parent auxotrophic cultures on MMA at the concentration used for hybridization. The yeast studied has a haplontic life cycle; it is hybridized and sporulates on the same starvation medium. Therefore, for each hybridized pair, the exposure time on SPA was adjusted in order to prevent sporulation. After 48–72 h, the prototrophic hybrid colonies grown on MMA at 28°C were additionally cloned on the same medium, streak-inoculated onto MEA medium for growth and sporulation, and incubated at 25°C for 48–72 h. For the hybrids with the involvement of the strains CBS 1043 and SPK571, the hybridization time

on SPA did not exceed 2–3 h; after that, the mixed culture was immediately cloned on MMA. The grown hybrid colonies sporulated on the same medium and were therefore analyzed without the subsequent transfers. Homothallism was revealed by the alteration of the coloration of the MEA-grown colonies on exposure to iodine [23].

PCR analysis. The yeast DNA was isolated with the Genomic DNA Purification Kit (Fermentas, Lithuania). The polymerase chain reaction was performed using the Tercyc^{TM} DNA amplifier (DNA-Technology, Russia). The DNA was amplified with the RAPD primers OPA-01 (5'-CAGGCCCTTC), OPA-04 (5'-AATCGGGCTG), OPA-9 (5'-GGGTAACGCC), and OPA-11 (5'-CAATCGCCGT) in 30 μL of the buffer containing 3 mM $MgCl_2$, 0.3 mM of each dNTP, 50 pmol of each primer, 1.25 U of active *Taq*-polymerase (Syntol, Russia), and 20–200 ng of the genomic DNA analyzed. Forty-five PCR cycles were accomplished in the following mode: DNA denaturation, 94°C, 1 min; primer annealing, 36°C, 1 min; DNA synthesis, 72°C, 2 min. The amplified DNA fragments were separated in 1.2% agarose gel at 55–60 V in 0.5× TBE buffer for 2–3 h. The gel was stained with ethidium bromide for 1–2 h, washed in distilled water, and photographed under UV illumination.

Phylogenetic analysis. The interstrain relationships were established by comparing the profiles of the PCR products amplified with the RAPD primers OPA-01, OPA-04, OPA-09, and OPA-11. The dendrograms based on the matrix of differences in the PCR profiles with these primers were constructed using the Neighbor-Joining algorithm implemented in the TREECON software package [24]. The bootstrap indices determining the statistical significance of group isolation were determined for 1000 pseudo replicas. The strain *Saccharomyces cerevisiae* S288c was used as the outgroup.

Pulsed-field electrophoresis of the chromosomal DNA. Chromosomal preparations were obtained as described previously [25]. The samples were placed into the slits of 1% agarose gel. 0.5× TBE cooled to 14°C was used as a buffer. Electrophoresis was carried out on the CHEF-DR III apparatus (Bio-Rad, United States) at 50 V in the following mode: (1) 48 h with a field switching time of 2400 s; (2) 70 h with a field switching time of 3000 s; and (3) 24 h with a field switching time of 3300 s. After electrophoresis, the gel was stained with ethidium bromide, washed in distilled water, and photographed. The strains *Saccharomyces cerevisiae* YNN 295 and *Sch. pombe* 972 h^- (Bio-Rad, United States) with known chromosomal size and order were used as the karyotypic standards.

RESULTS

Genetic study of the geographic collection of *Sch. pombe* strains from CBS. The *Schizosaccharomyces* spores containing a starch-like substance in the cell wall are stained black upon exposure to iodine. Using this criterion, the strains CBS 356, CBS 1043, CBS 5557, CBS 5680, and CBS 5682 were identified as homothallic; CBS 357 did not exhibit black coloration, but in hybridizations with the testers of both mating types, this strain behaved as a homothallic one. The strains CBS 1057, CBS 1061, and CBS 2628 did not stain on exposure to iodine due to the absence of spores and were preliminarily identified as heterothallic.

Monosporic and monocellular clones were obtained from the sporulating homothallic strains and from heterothallic strains, respectively. Monospore clones with high ascospore viability were selected: 356-4B, 1043-1B, 5557-1A, 5680-2B, and 5682-2C with 75, 100, 100, 76, and 100% viability, respectively. In all the strains, the high induction rate was noted only for the *ura* and *ade* mutants. The homothallic auxotrophic mutants sporulated poorly. The spore viability was analyzed only in 1043-1B *ura6*, which was 81%.

The hybrids nos. 1–6 obtained during control intrastrain hybridizations formed numerous asci containing well-developed spores with 53 to 72% germination rate. Normal meiotic segregation of the control markers was characteristic of all intrastrain hybrids (Table 2).

All interstrain hybrids also sporulated abundantly, except hybrid no. 30; the asci normally contained four spores, whose size and shape were usually normal. However, with few exceptions, these ascospores isolated with the micromanipulator did not form colonies on a complete medium (Table 3).

Analysis of the random sample of the interstrain hybrid spores revealed all classes of segregants of the control markers, including double auxotrophic recombinants (Table 3). Nevertheless, the number of prototrophic clones was dominated.

Hybridological analysis of *Sch. kambucha* SPK571 and of *Sch. pombe* genetic lines. In strain SPK571, the monospore clones were isolated with a micromanipulator. Stable auxotrophic mutants *ade* and *his* were obtained by UV irradiation from the selected, well-sporulating clone of strain SPK571 with high ascospore viability (87%) and from the monocellular culture of strain 975 h^+. Strain 972 h^- had the *ade* auxotrophy.

We analyzed the following control intrastrain hybridizations: hybrid no. 7 of the *ade2/his4* genotype and hybrid no. 8 of the heterothallic yeasts 972 and 975 (descendants of the same strain) of the h^-*ade5*/h^+*his1* genotype (Table 2). In both cases, the hybrids were highly fertile with an ascospore viability of 73 and 85%, respectively. Meiotic segregation of the control markers was digenic. The observed decrease in the occurrence rate of tetratype tetrads might indicate a

Table 2. Genetic analysis of the intrastrain hybrids of the yeast *Sch. pombe*

Hybrid no.	Origin of the hybrids, genotypes	The number of isolated spores	Spore viability, %	Segregation* of the control markers AB:aB:Ab:ab**
1	1043 × 1043			
	ade1/ura6	69	53	9:5:6:7
2	5680 × 5680			
	ade1/ura5	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
7	571 × 571			
	ade2/his4	100	73	4P:4N:6T***
8	972 h^- × 975 h^+			
	ade5/his1	72	85	3P:3N:7T

* Segregation is shown on the sample of spores isolated with a micromanipulator.
** a, b: the auxotrophies of the first and second parent before the hybridization sign, respectively; A, B, prototrophies.
*** P, N, T: the parental (P), nonparental (N) ditype tetrads and the tetratype (T) tetrads.

linkage of the marker genes with the centromeres of their chromosomes.

The complementary auxotrophic mutants of SPK571 and 972 h^- were hybridized with each other, with mutants of the type culture *Sch. pombe* CBS 356, as well as with mutants of all the CBS strains of this species, previously studied by us. Strains CBS 2628 *ade1* and CBS 1057 *ade1* formed hybrids only with 975 h^+*his1*, which indicated that they have the h^- mating type. All of the interstrain hybrids nos. 31, 32, 34–46, as well as most hybrids obtained by us in hybridizations of yeasts of different geographic origin (Table 3), had an extremely low ascospore viability (0–6%). Only in the case of hybrids no. 33 and no. 47, this value was higher: 12 and 15%, respectively. Low ascospore viability (0.9%) was also reported in the literature [26] when the strains CBS 1043, CBS 5680, and CBS 1057 were hybridized with *Sch. pombe* genetic lines.

Using the micromanipulator, we isolated approximately one hundred spores from each hybrid. Only analysis of the random spore sample revealed viable colonies. The control markers showed deviation from digenic segregation. Nevertheless, all types of meiotic segregants were present (Table 3). Only hybrid no. 39 had no double auxotrophic recombinants or segregants of the same parent type. Recombination of the control markers indicated that strain SPK571, the genetic lines, the type culture *Sch. pombe* CBS 356, and other natural isolates studied by us were closely related and that all these yeasts belonged to the same species—*Sch. pombe*. It is noteworthy that strain SPK571 did not belong to the independent species

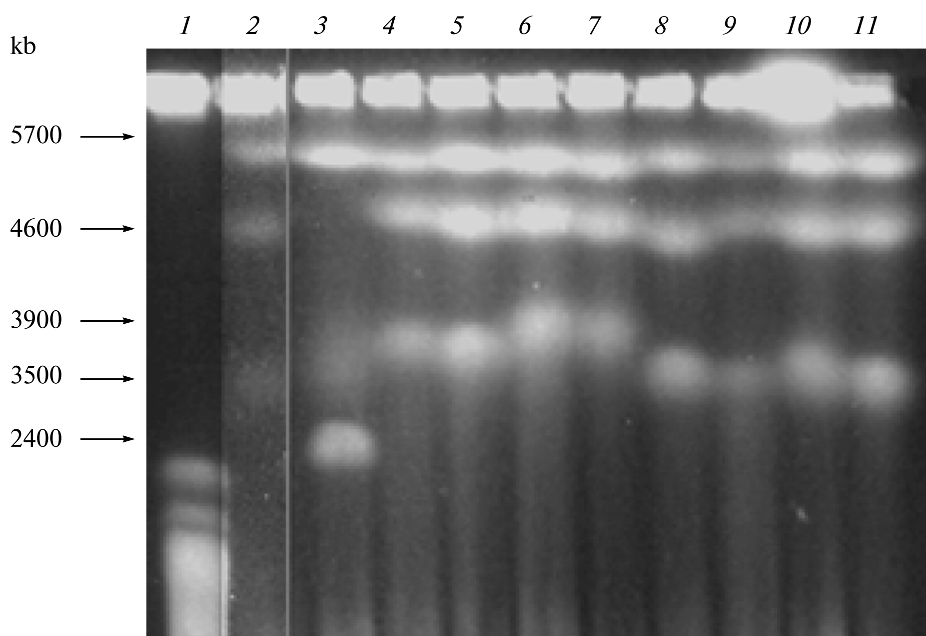

Fig. 1. Pulsed-field gelelectrophoresis of the chromosomal DNA of the yeast *Schizosaccharomyces pombe*: CBS 7264 (972 h^-) (*2*), CBS 356 (*3*), CBS 357 (*4*), CBS 1057 (*5*), CBS 1061 (*6*), CBS 5557 (*7*), CBS 1043 (*8*), CBS 2628 (*9*), CBS 5680 (*10*), and CBS 5682 (*11*). The chromosomal order and size (kb) are given according to the standards of *Schizosaccharomyces cerevisiae* YNN 295 and *Schizosaccharomyces pombe* 972 h^- (lanes *1* and *2*, respectively).

previously termed *Sch. kambucha*. This agrees well with 98% identity of the nucleotide sequences of the of the *mat2* and *mat3* silent copies in the mating type locus of *Sch. pombe* and *Sch. kambucha* [17]. Obviously, death of the hybrid spores in hybridizations of *Sch. kambucha* with *Sch. pombe* first revealed by the authors [16] agrees with the phenomenon of ascospore death revealed by us in the hybrids of different natural strains of *Sch. pombe* from the CBS collection. Belonging of CBS 357, CBS 1061, and CBS 5557 to the same species was confirmed by their high DNA/DNA reassociation (92–98%) with the type culture CBS 356 [27].

Pulsed-field gelelectrophoresis of native chromosomal DNA of the yeast *Sch. pombe*. Karyotypic analysis of 10 strains of *Sch. pombe* was carried out (Fig. 1). The size and order of the chromosomal bands were established according to the karyotypic standards of *Saccharomyces cerevisiae* YNN295 and *Sch. pombe* 972 h^- (lanes *1* and *2*). The chromosomal DNA of all the *Sch. pombe* strains studied formed three bands ranging in size from 2200 to 5700 kb. Despite the same number of chromosomes, the size of individual chromosomal bands varied significantly from strain to strain (lanes *2–11*). The size of the lowest chromosomal band was the most variable (from 2400 to 3900 kb). Almost every one of the ten strains studied had a specific chromosomal profile. The greatest differences were noted in the type strain CBS 356, whose molecular karyotype was characterized by the presence of the 2400-kb chromosomal band (lane *3*). All of the other strains had a considerably larger size of the lower chromosomal band: 3500–3900 kb (Fig. 1).

PCR analysis. It is known that RAPD markers have multiple localization in the yeast genome, which makes it possible to compare a large number of polymorphic loci and to reveal genetic divergence of the strains.

Further study of *Sch. pombe* genomes was carried out by PAPD-PCR using the primers OPA-01, OPA-04, OPA-09, and OPA-11. The strain *S. cerevisiae* S288c, whose PCR profiles differed significantly from the patterns of *Sch. pombe* strains in both the number and the size of amplified bands (Fig. 2, lane *1*), was used as the control. *Sch. pombe* strains had different PCR profiles when the four RAPD primers were used. The greatest differences in the PCR profiles were obtained with the primers OPA-01 and OPA-04 (Figs. 2a, 2b; lanes *2–10*). The differences concerned both the sizes of amplified DNA fragments and the presence or absence of individual major and minor bands. As in the case of molecular karyotypes, each of the *Sch. pombe* strains studied had a unique pattern. The PCR profile of the type culture CBS 356 was characterized by the greatest differences (Fig. 2, lane *2*).

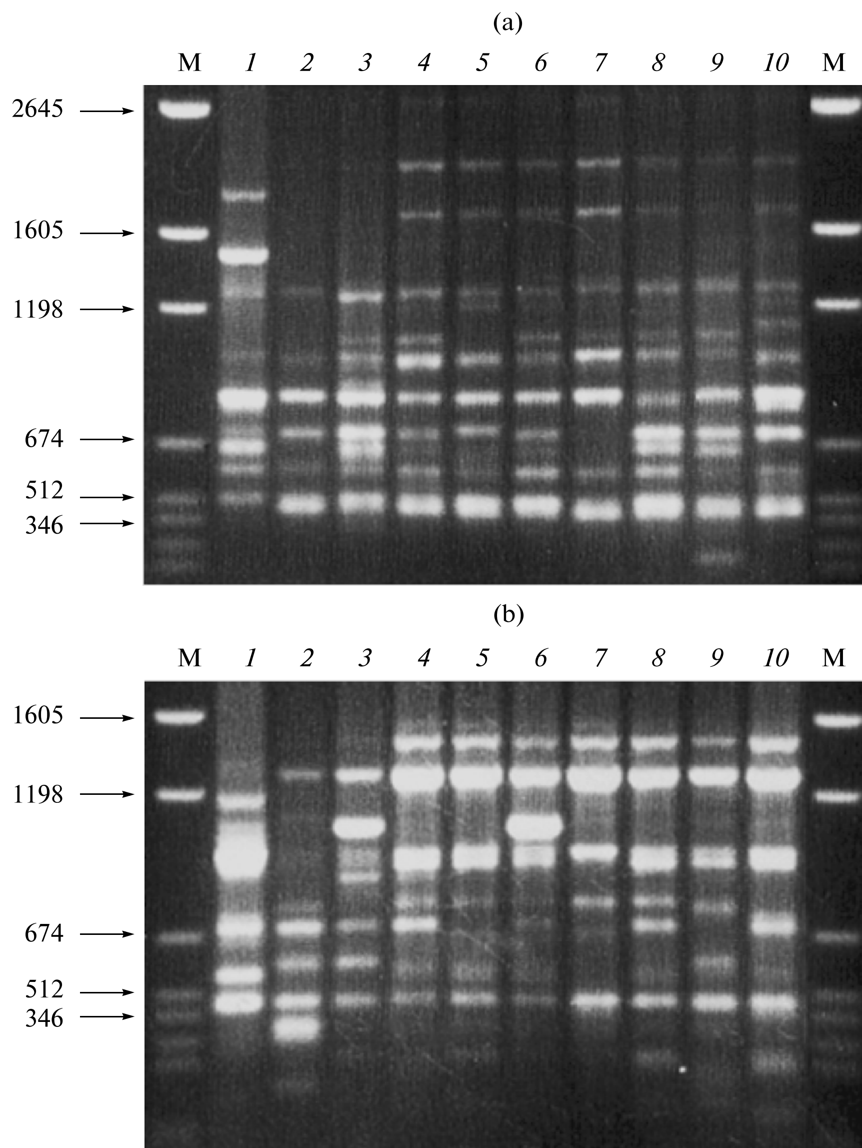

Fig. 2. PCR analysis of *Schizosaccharomyces pombe* strains using the RAPD-primers OPA-01 (a) and OPA-04 (b): CBS 356 (*2*), CBS 357 (*3*), CBS 1057 (*4*), CBS 1061 (*5*), CBS 5557 (*6*), CBS 1043 (*7*), CBS 2628 (*8*), CBS 5680 (*9*), and CBS 5682 (*10*). The control strain: *Saccharomyces cerevisiae* S288c (*1*). M is the molecular weight marker (bp).

Based on the data of PCR analysis with four RAPD primers, a binary matrix of differences was compiled and a matrix-based dendrogram was constructed (Fig. 3). *Sch. pombe* strains formed a separate cluster in relation to the test strain *S. cerevisiae* S288c. Two groups could be identified inside the cluster, each of which, in turn, included two subgroups. The type culture CBS 356 occupied a special position in the tree. No correlation between the origin of the strains (Table 1) and their arrangement in the tree was found (Fig. 3). Thus, the representatives of different geographic populations of *Sch. pombe* had genomic differences in both the chromosomal size and RAPD markers.

DISCUSSION

First of all, it should be noted that the heterogeneity of the genus *Schizosaccharomyces* and assignment to it of solely *Sch. pombe* species have repeatedly been highlighted in the literature [8, 28–30]. We tend to consider this genus (*Schizosaccharomyces* sensu stricto) as a polytypic genus in statu nascendi. It is expedient to draw attention to the low statistical sup-

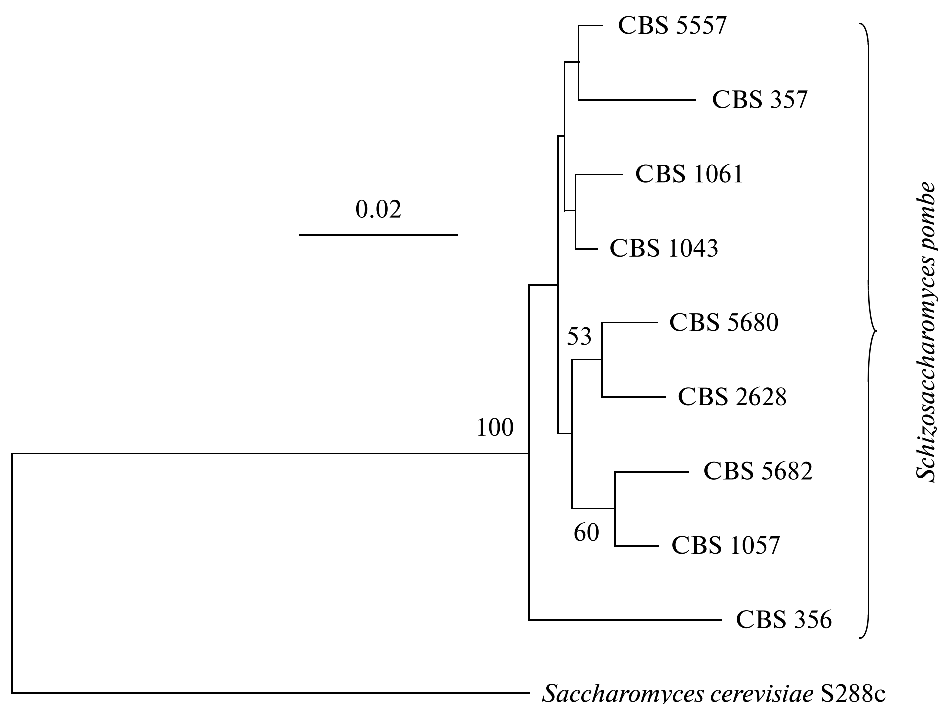

Fig. 3. Dendrogram of the relatedness between *Schizosaccharomyces pombe* strains based on the matrix of differences in the PCR profiles with the RAPD primers OPA-01, OPA-04, OPA-09, and OPA-11. The strain *Saccharomyces cerevisiae* S288c was used as the outgroup. The bootstrap values >50% are given.

port of the phylogenetic relatedness of the taxa of *Sch. pombe,* to *Sch. cryophilus*, *Sch. japonicus,* and *Sch. octosporus* included in the cluster *Schizosaccharomyces* [8, 31–33].

Earlier, it was shown that the yeast *Sch. pombe* is not hybridized with other recognized species of this genus [34]. According to the conception of the genetic genus developed by us for the ascomycetous yeasts, the species of the same genus share a common mating type system that enables them to cross in any combination. Nevertheless, genetic genera combine biological species whose hybrids are completely sterile. The existence of genetic genera and biological species has been demonstrated for *Saccharomyces, Williopsis, Arthroascus, Galactomyces, Kluyveromyces* sensu stricto, and *Metchnikowia* sensu stricto [34–41].

Actually, the genus represented only by the species *Sch. pombe* is not unambiguously monotypic as it consists of partly genetically isolated populations. The sterility of interstrain hybrids and meiotic recombination of the control markers in them indicate that the divergent populations of *Sch. pombe* are species in statu nascendi. The literature data [15] and our findings on the karyotypic diversity of *Sch. pombe* of different geographic origin agree well with the phenomenon of spore killing in interstrain hybridizations. It is universally recognized that extensive chromosomal rearrangements, which were revealed in *Sch. pombe* [15], always lead to hybrid sterility.

Description of the taxa whose rank is lower than the species level (varieties) is possible in the taxonomy of yeasts. According to our experience with the yeasts *Arthroascus, Kluyveromyces, Saccharomyces*, and *Zygowilliopsis* [38, 40, 42–45], the populations of the yeasts whose hybrids are characterized by low spore viability (normally 0–20%) but have meiotic recombination of the control parent markers may be considered as varieties. With such an approach, all the eleven *Sch. pombe* strains studied by us are the representatives of different taxonomic varieties. Eight strains already have different species names (Table 1). It is expedient to renew them as the names of varieties after the isolation of the corresponding additional strains. Taking into account that all the eleven randomly chosen strains represented divergent populations, we can also predict with high probability the status of varieties for the remaining synonyms of the species *Sch. pombe: Sch. acidodevoratus* Tschalenko 1941 (VKM Y-646^T), *Sch. formosensis* var. *akoensis* Nakazawa 1914 (CBS 1044^T), *Sch. formosensis* var. *tapaniensis* Nakazawa 1914 (CBS 1062^T = VKM Y-649), *Sch. mosquensis* Shcherbakov et Popova 1934 (VKM Y-653^T), *Sch. pombe* var. *iotensis* Sakaguchi (CBS 2776), *Sch. pombe* var. *ogasawaraensis* Sakaguchi et Otani (CBS 2775), *Sch. santawensis* Nakazawa 1914 (CBS 1063^T = VKM Y-664), and *Sch. pinan* Nakazawa 1919 (CBS 355 = VKM Y-657).

Table 3. Genetic analysis of the interstrain hybrids of the yeast *Sch. pombe*

Hybrid no.	Origin of the hybrids, genotypes	Number of isolated spores	Spore viability, %	Segregation* of the control markers AB:aB:Ab:ab**	Hybrid no.	Origin of the hybrids, genotypes	Number of isolated spores	Spore viability, %	Segregation of the control markers AB:aB:Ab:ab
9	5680 × 5682				29	5682 × 357			
(a)	*ade1/ura4*	48	0	85:11:51:4		*ura4/ade1*	51	2	140:45:151:69
(b)	*ura5/ade16*	101	1	129:49:15:38					
10	2628 × 5680				30	1061 × 5557			
	ade1/ura5	119	0	130:13:18:7		*ade4/ura7*	–	–	41:104:18:101
11	5680 × 1043				31	972 h^{-} × 571			
(a)***	*ade1/ura6*	188	21	18:6:10:5		*ade5/his4*	104	0	196:12:48:10
b)	*ura5/ade1*	52	8	57:56:44:43					
12	357 × 1057				32	972 h^{-} × 356			
	ura2/ade1	96	0	122:27:55:52		*ade5/met5*	100	2	145:20:108:17
13	356 × 5557				33	972 h^{-} × 5557			
	ade3/ura7	132	0	65:13:10:16		*ade5/ura3*	100	12	154:33:109:25
14	356 × 1043				34	972 h^{-} × 5682			
	ura16/ade1	100	0	198:36:13:77		*ade5/ura4*	96	2	82:10:30:19
15	356 × 5680				35	972 h^{-} × 5680			
(a)	*ura16/ade1*	111	3	94:61:11:7		*ade5/ura5*	72	2	302:26:209:9
(b)	*ade12/ura5*	24	0	187:13:12:11					
16	356 × 5682				36	972 h^{-} × 357			
	ura16/ade14	92	0	76:105:17:8		*ade5/ura2*	88	0	170:35:41:28
17	5680 × 357				37	972 h^{-} × 1043			
(a)	*ade1/ura2*	24	0	54:86:58:63		*ade1/ura6*	84	0	159:14:39:26
(b)	*ura5/ade1*	100	0	63:69:59:64					

Table 3. (Contd.)

Hybrid no.	Origin of the hybrids, genotypes	Number of isolated spores	Spore viability, %	Segregation* of the control markers AB:aB:Ab:ab**	Hybrid no.	Origin of the hybrids, genotypes	Number of isolated spores	Spore viability, %	Segregation of the control markers AB:aB:Ab:ab
18	5680 × 5557				38	975 h^+ × 2628			
	ura5/ade3	101	3	36:38:27:42		*his1/ade1*	68	2	134:29:16:34
19	1043 × 357				39	975 h^+ × 1057			
	ade1/ura2	100	0	217:2:17:11		*his1/ade1*	168	0	443:517:0:0
20	1043 × 5557				40	571 × 356			
	ade1/ura7	129	0	58:102:15:87		*ade2/ura7*	100	3	65:100:23:13
21	2628 × 5557				41	571 × 5557			
	ade7/ura7	92	0	168:6:37:30		*ade2/ura3*	100	0	86:68:37:15
22	5557 × 357				42	571 × 5682			
(a)	*ura7/ade1*	116	4	90:114:59:62		*ade2/ura4*	100	2	50:49:62:24
(b)	*ade3/ura2*	38	0	53:76:40:66					
23	5557 × 1057				43	571 × 568			
	ura7/ade1	57	0	186:0:113:20		*ade2/ura5*	100	0	82:23:64:14
24	2628 × 357				44	571 × 357			
	ade7/ura2	88	0	55:14:28:33		*ade2/ura2*	100	6	57:106:82:43
25	2628 × 1043				45	571 × 1043			
	ade7/ura6	100	0	154:31:34:33		*ade2/ura6*	168	0	484:528:15:17
26	2628 × 5682				46	571 × 2628			
	ade7/ura4	100	0	42:18:138:18		*his4/ade*	100	0	145:76:71:24
27	5682 × 5557				47	571 × 1057			
	ade14/ura7	70	10	97:60:66:3		*his4/ade1*	100	15	92:92:57:26
28	5682 × 1043								
	ura4/ade1	60	7	171:27:48:27					

* Segregation is shown on a random sample of spores obtained after treatment of the sporulating culture with ethanol.
** a, b: the auxotrophies of the first and second parent before the hybridization sign, respectively; A, B, prototrophies.
*** In this case, the spores were isolated with a micromanipulator.

Undoubtedly, the exceptionally unusual yeast *Sch. pombe* is a promising subject for studies in the field of evolutionary and taxonomic genetics.

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