Typing of *Saccharomyces cerevisiae* Clinical Strains by Using Microsatellite Sequence Polymorphism

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It seems that *S. cerevisiae*, which was thought for about 30 years to be a nonpathogenic yeast, should now be considered an opportunistic pathogen. In this study, we estimated the discrimination ability of the microsatellite sequence amplification technique within a sample of clinical and reference *S. cerevisiae* strains and *S. boulardii* reference strains.

Saccharomyces cerevisiae is a saprophyte of the digestive, respiratory, and genitourinary tracts (2-4, 17, 18, 25). However, with the progress in immunosuppressive therapies, there has been an increasing number of reports on yeast systemic infection cases involving *Saccharomyces* strains, particularly *S. cerevisiae*. The epidemiology of infections caused by *Saccharomyces* is still unknown. Some authors suggested a correlation between fungemia caused by *Saccharomyces* and treatment (24). Moreover, *S. cerevisiae* is usually considered to be responsible for infections. Nevertheless, some authors have suggested the role of other *Saccharomyces* species in systemic infections. Some of these infections may be due to treatment of diarrhea by *S. boulardii* (9, 20). It would thus be necessary to develop molecular markers for distinguishing various genotypes within *S. cerevisiae*.

Some epidemiological studies have shown intraspecific diversity within *S. cerevisiae* (11, 12). The present retrospective multicenter study exhibited polymorphism in microsatellite sequences of 69 clinical strains of *S. cerevisiae* by comparison to *S. cerevisiae* and *S. boulardii* reference strains. The aim of this work was to study genotypic polymorphism within clinical strains of *S. cerevisiae* compared to *S. boulardii* reference strains.

MATERIALS AND METHODS

In this study, 77 strains were tested; 69 came from different French medical centers (Table 1). These strains were isolated from different samples (Table 1). In parallel, five *S. cerevisiae* and three *S. boulardii* reference strains were tested (Table 1).

Yeast DNA was purified according to the protocol published by Querol et al. (22). The five microsatellite sequences used in our study have been previously described (10). These sequences are distributed in the *S. cerevisiae* genome (Table 2). PCR amplification was performed according to the protocol described by Durand et al. (8).

After heat denaturation, PCR fragments were separated by electrophoresis on a vertical polyacrylamide gel including formamide in the loading buffer.

The data were analyzed by factorial correspondence analysis with the PRAXIS-p.c., version 2.0, software package (Praxème, R&D, Biométrie, C.N.R.S., Montpellier, France) (1, 6).

Factorial correspondence analysis was presented as a plane projection of the

two most informative axes accounting for the genetic structure of the electrophoretic types. Allelic frequencies with each locus were calculated with the Genepop, version 1.2, software program (23).

RESULTS AND DISCUSSION

The microsatellite sequence polymorphism analysis gave reproducible results. Each locus tested was polymorphic, with 6, 11, 6, 6, and 5 different alleles for the microsatellite sequences of genes YKL 172W, YLR 177W, YKL 139W, YKR 072C, and YDR 289C, respectively. The fact that certain strains presented two different alleles for a given locus increased the number of patterns observed. In the present study, 12 pairs of different alleles were detected for the YKL 172W gene, 18 for YLR 177W, 11 for YKL 139W, 8 for YKR 072C, and 6 for YDR 289C. A predominant pair of alleles was found in microsatellite sequences of genes YKR 172W, YKR 072C, and YDR 289C, whereas for genes YLR 177W and YKL 139W, the allele pairs were distributed in a homogeneous way without any of them dominating (Table 3).

In the present work, 34 different alleles were observed and generated 64 distinct electrophoretic types. Among these 64 electrophoretic types, 58 represented a single strain, three represented two strains (electrophoretic types 26, 30, and 53), one represented four strains (electrophoretic type 14), one represented three strains (electrophoretic type 64), and one represented five strains which were isolated from the same patient (electrophoretic type 54). On the factorial correspondence analysis, electrophoretic types are projected in the most informative plane defined by axes 1 and 2, which explained 21.29% of the overall genetic variability.

The electrophoretic type projection exhibited three distinct clusters (Fig. 1). Cluster I included only clinical strains of *S. cerevisiae*. Cluster II included reference strains of *S. cerevisiae*, and cluster III contained reference strains of *S. boulardii*. These three clusters were separated by axis 1, which was the most informative. The loci responsible for the structure of the sample were highlighted by the allelic frequencies indexed in Table 3. Allele pairs responsible for the structure of the sample were the least frequent (i.e., 177-30, 177-31, 13-32, 072-33, and 289-34).

Genotype differences between the electrophoretic types of

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TABLE 1. Characteristics of the *Saccharomyces* strains studied

Strain ^a	Origin ^b	Sample ^c	Species	Sampling date (mo/yr)	ET ^d
IVP 406	LABM Montpellier	Feces	<i>S. cerevisiae</i>	05/1999	1
IVP 483	LABM Montpellier	Feces	<i>S. cerevisiae</i>	03/2000	2
IVP 701	LABM Montpellier	Vagina	<i>S. cerevisiae</i>	09/2000	3
IVP 712	LABM Montpellier	Feces	<i>S. cerevisiae</i>	10/2000	4
IVP 747	LABM Montpellier	Vaginal	<i>S. cerevisiae</i>	12/2000	5
IVP 800	LABM Montpellier	Feces	<i>S. cerevisiae</i>	05/2000	6
IVP 1417	CHU Montpellier	Oropharynx	<i>S. cerevisiae</i>	04/1990	7
IVP 1725	CHU Montpellier	Oropharynx	<i>S. cerevisiae</i>	05/1992	8
IVP 4025	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	9
IVP 4026	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	10
IVP 4027	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	11
IVP 4028	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	12
IVP 4030	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	13
IVP 4031	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	14
IVP 4032	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	14
IVP 4033	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	14
IVP 4035	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	15
IVP 4036	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	16
IVP 4037	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	17
IVP 4038	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	18
IVP 4039	LABM Montpellier	Unknown	<i>S. cerevisiae</i>	Unknown	19
IVP 4068	CHU Paris	Feces	<i>S. cerevisiae</i>	01/1995	20
IVP 4069	CHU Paris	Feces	<i>S. cerevisiae</i>	01/1995	21
IVP 4070	CHU Paris	Feces	<i>S. cerevisiae</i>	01/1995	22
IVP 4151	CHU Montpellier	Blood	<i>S. cerevisiae</i>	Unknown	23
IVP 4153	CHU Montpellier	Blood	<i>S. cerevisiae</i>	Unknown	24
IVP 4155	CHU Montpellier	Blood	<i>S. cerevisiae</i>	Unknown	25
IVP 4171	CHU Paris	Blood	<i>S. cerevisiae</i>	04/1997	26
IVP 4172	CHU Paris	Blood	<i>S. cerevisiae</i>	04/1997	27
IVP 4173	CHU Paris	Blood	<i>S. cerevisiae</i>	04/1997	26
IVP 4174	CHU Paris	Blood	<i>S. cerevisiae</i>	04/1997	28
IVP 4175	CHU Paris	Blood	<i>S. cerevisiae</i>	04/1997	29
IVP 4176	CHU Paris	Blood	<i>S. cerevisiae</i>	04/1997	30
IVP 4177	CHU Paris	Blood	<i>S. cerevisiae</i>	04/1997	30
IVP 4178	CHU Paris	Blood	<i>S. cerevisiae</i>	04/1997	14
IVP 4179	CHU Paris	Blood	<i>S. cerevisiae</i>	04/1997	31
IVP 4274	CHU Montpellier	Oropharynx	<i>S. cerevisiae</i>	07/1998	32
IVP 4277	CHU Montpellier	Oropharynx	<i>S. cerevisiae</i>	10/2000	33
IVP 4278	CHU Montpellier	Oropharynx	<i>S. cerevisiae</i>	10/2000	34
IVP 4297	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	11/2001	35
IVP 4298	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	11/2001	36
IVP 4299	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	11/2001	37
IVP 4300	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	11/2001	38
IVP 4301	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	11/2001	39
IVP 4302	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	11/2001	40
IVP 4303	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	11/2001	41
IVP 4304	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	11/2001	42
IVP 4305	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	11/2001	43
IVP 4308	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	03/2002	44
IVP 4309	CHU Bordeaux	BAL	<i>S. cerevisiae</i>	03/2002	45
IVP 4310	CHU Bordeaux	Oropharynx	<i>S. cerevisiae</i>	03/2002	46
IVP 4311	CHU Bordeaux	Oropharynx	<i>S. cerevisiae</i>	03/2002	47
IVP 4312	CHU Rennes	Feces	<i>S. cerevisiae</i>	03/2002	46
IVP 4313	CHU Rennes	Feces	<i>S. cerevisiae</i>	03/2002	48
IVP 4314	CHU Rennes	BAL	<i>S. cerevisiae</i>	03/2002	49
IVP 4315	CHU Marseille	Vagina	<i>S. cerevisiae</i>	04/2002	50
IVP 4316	CHU Marseille	Vaginal	<i>S. cerevisiae</i>	04/2002	51
IVP 4317	CHU Marseille	Oropharynx	<i>S. cerevisiae</i>	04/2002	52
IVP 4318	CHU Marseille	Blood	<i>S. cerevisiae</i>	04/2002	53
IVP 4319	CHU Marseille	Blood	<i>S. cerevisiae</i>	04/2002	53
IVP 4320	CHU Marseille	Blood	<i>S. cerevisiae</i>	04/2002	54
IVP 4321	CHU Marseille	Blood	<i>S. cerevisiae</i>	04/2002	54
IVP 4323	CHU Marseille	Feces	<i>S. cerevisiae</i>	04/2002	55
IVP 4324	CHU Marseille	Feces	<i>S. cerevisiae</i>	04/2002	54
IVP 4325	CHU Marseille	Urinary sample	<i>S. cerevisiae</i>	04/2002	54
IVP 4326	CHU Marseille	BAL	<i>S. cerevisiae</i>	04/2002	54
IVP 7044	CHU Toulouse	Unknown	<i>S. cerevisiae</i>	01/1995	56
IVP 7046	CHU Toulouse	Unknown	<i>S. cerevisiae</i>	09/1994	57
IVP 7047	CHU Toulouse	Unknown	<i>S. cerevisiae</i>	10/1994	58
IVP 4266	CLIB 157	Wine	<i>S. cerevisiae</i>	04/1998	59
IVP 4267	CLIB 193	Unknown	<i>S. cerevisiae</i>	04/1998	60
IVP 4268	CLIB 194	Unknown	<i>S. cerevisiae</i>	04/1998	61
IVP 4269	CLIB 227	Beer	<i>S. cerevisiae</i>	04/1998	62
IVP 4270	CLIB 338	Plant	<i>S. cerevisiae</i>	04/1998	63
IVP 4271	UL G84F88I90	Biocodex Laboratoires	<i>S. boulardii</i>	04/1998	64
IVP 4272	UL 97/7	Biocodex Laboratoires	<i>S. boulardii</i>	04/1998	64
IVP 4273	UL 97/34	Biocodex Laboratoires	<i>S. boulardii</i>	04/1998	64

^a IVP, Immuno-viro-parasitologie; ATCC, American Type Culture Collection; CLIB, Collection Levures d'Intérêt Biologiques.^b LABM, Laboratoire d'Analyses Biologiques et Médicales; CHU, Centre Hospitalier Universitaire.^c BAL, bronchoalveolar lavage fluid.^d ET, electrophoretic type.

TABLE 2. Characteristics of the five polymorphic microsatellite loci tested in this study

Gene	Sequence repeat	Sequence of upper primer (5' → 3')	Sequence of lower primer (5' → 3')
YKL 172W	GAA	CAGGACGCTACCGAAGCTCAAAAG	ACTTTTGGCCAATTTCTCAAGAT
YKR 072C	GAC	AGATACAGAAGATAAGAACGAAAA	TTATTGATGCTTATCTATTATACC
YKL 139W	AAT	AAGCGTCCTAACATACTATCCACC	ATTTC AATTGGCTATATATCCTTA
YLR 177W	CAG	CTTAAACAACAGCTCCCAA	ATGAATCAGCGCATCAGAAAT
YDR 289C	ATC	AATTGCTGTCAATTGGATCTAT	ATTATTCTACGTATGAAGTG

cluster I were investigated with a second factorial correspondence analysis (data not shown). Genotypes were not linked to a geographical origin or anatomic site. No conclusions can thus be drawn at present, but it would be interesting to conduct a further study with a larger sample of isolates.

In this study the sample assessed was interesting, related exclusively to clinical *S. cerevisiae* strains. This sample should be increased, but it is very difficult to obtain strictly clinical

strains of *S. cerevisiae*, which explains why the present sample was limited in size and spatiotemporally heterogeneous.

Sixty-four electrophoretic types for the 77 strains were found by the microsatellite sequence polymorphism technique. These results are in agreement with those of Zerva et al. by using restriction fragment length polymorphism (26). Joly et al. (11) with randomly amplified polymorphic DNA and Duarte et al. (7) with multilocus enzyme electrophoresis on *S. cerevisiae*

FIG. 1. First factorial correspondence analysis plane projection in two informative axes of all electrophoretic types observed. Axes 1 and 2 represent 11.73% and 9.56% of the overall variability, respectively. Cluster I includes all *S. cerevisiae* clinical strains, cluster II includes *S. cerevisiae* reference strains, and cluster III includes *S. boulardii* reference strains. Clusters are separated on axis 1, which is the most informative.

TABLE 3. Allelic frequencies for the five loci tested^a

Locus	Frequency											
	1	2	3	4	5	6	7	8	9	10	11	NA.
YKR 172W	0.48	0.14	0.17	0.06	0.06	0.04						0.05
YKR 177 W	0.08	0.07	0.03	0.07	0.11	0.15	0.20	0.06	0.14	<0.01	<0.01	0.08
YKL 139 W	0.02	0.26	0.32	0.26	0.05	0.04						0.05
YKR 072 C	<0.01	0.72	0.07	0.01	0.01	0.10						0.09
YDR 289 C	0.09	0.75	0.04	0.01	0.10							0.01

^a Alleles 1 to 11 were assigned numbers in order according to their increasing anodal mobility. NA., not amplified. Alleles responsible for the structure of the analysis (Fig. 1) were the least frequent.

found 48 types for 54 isolates and 32 types for 35 studied strains, respectively. Hennequin et al. (10) differentiated 52 types for 91 strains tested by the microsatellite technique. This could be explained by the high homogeneity of the clinical strain samples studied. In the work of Lewicka et al. (12), only 22 types for 52 strains by multilocus enzyme electrophoresis were observed. However, it appears that in all of these studies, there was high diversity in the clinical and nonclinical *S. cerevisiae* strains.

In the present study, the clinical strains of *S. cerevisiae* (cluster I) seemed to have genotypes distinct from those of the *S. cerevisiae* reference strains studied (cluster II). This is in agreement with the work of Clemons et al. (5) in which the clinical and nonclinical strains showed different degrees of virulence. These results are of epidemiological importance, because in the present work, environmental strains had electrophoretic profiles which differed from those of strains isolated from patients. The genotypes of the *S. cerevisiae* strains responsible for infection and those isolated from the environment were very different. Microsatellite typing also revealed a genotypic difference between the *S. cerevisiae* and *S. boulardii* strains tested. This is in agreement with Mallié et al. (13) and McFarland et al. (15), who differentiated *S. cerevisiae* from *S. boulardii* by multilocus enzyme electrophoresis.

However, some authors such as Molnar et al. (16), Perapoch et al., and McCullough et al. demonstrated, by randomly amplified polymorphic DNA and restriction fragment length polymorphism (14, 19), respectively, that *S. cerevisiae* and *S. boulardii* have identical genetic profiles. Moreover, Hennequin et al. (10) found the same profile for clinical *Saccharomyces* strains and *S. boulardii* strains. Nevertheless, no variations in the genotype of *S. boulardii* have ever been observed (10, 21), which is in agreement with the results of the present study. Indeed, the same electrophoretic type was found for the three *S. boulardii* reference strains tested. This work exhibited a difference between clinical strains of *S. cerevisiae* (cluster I) and reference strains of *S. boulardii* (cluster III). These results should be confirmed by a comparative study on a larger sample of *S. cerevisiae* and *S. boulardii* strains.

By the description of 64 different electrophoretic types for the 77 strains tested, the microsatellite sequence polymorphism technique highlighted significant intraspecific diversity within *S. cerevisiae* for both the clinical and reference strains. Microsatellite sequence polymorphism is a powerful molecular typing tool. The present work demonstrated a difference between clinical *S. cerevisiae* and *S. boulardii* strains, suggesting that *S. boulardii* was not responsible for the systemic fungal infections observed within the patients studied. It would be

useful to carry out a comparative study with a broader sample of *S. cerevisiae* and *S. boulardii* in order to determine their roles in human pathological infections. This could help to clarify the transmission mechanisms of these fungi and identify the contamination risk factors so that more effective prevention measures can be implemented.

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