Genetic Characterization of Pathogenic *Saccharomyces cerevisiae* Isolates

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ABSTRACT

Saccharomyces cerevisiae isolates from human patients have been genetically analyzed. Some of the characteristics of these isolates are very different from laboratory and industrial strains of *S. cerevisiae* and, for this reason, stringent genetic tests have been used to confirm their identity as *S. cerevisiae*. Most of these clinical isolates are able to grow at 42°, a temperature that completely inhibits the growth of most other *S. cerevisiae* strains. This property can be considered a virulence trait and may help explain the presence of these isolates in human hosts. The ability to grow at 42° is shown to be polygenic with primarily additive effects between loci. *S. cerevisiae* will be a useful model for the evolution and genetic analysis of fungal virulence and the study of polygenic traits.

SACCHAROMYCES cerevisiae has been used in the preparation of food and drink for centuries and is even consumed as a nutritional supplement by some individuals. This long association with mankind and the apparent rarity of *S. cerevisiae* infections has led to the perception that all isolates of this yeast are nonpathogenic. However, an examination of the literature since 1980 reveals occasional reports of infections that were attributed to *S. cerevisiae* (AUCOTT *et al.* 1990; CIMOLAI *et al.* 1987; DOUGHERTY and SIMMONS 1982; ENG *et al.* 1984; ESCHETE and WEST 1980; KIEHN *et al.* 1980; MANZELLA *et al.* 1989; NIELSEN *et al.* 1990; SETHI and MANDELL 1988; TAWFIK *et al.* 1989). These infections, which include sterile body sites and invasive infections, occurred primarily, although not exclusively, in severely debilitated, traumatized or immune-deficient patients.

These reports call into question the assumption that all *S. cerevisiae* isolates are completely benign. However, in none of these reported cases of *S. cerevisiae* infections has there been a thorough characterization of the infecting microorganism. First, identification was based on tests which cannot distinguish between species within the genus *Saccharomyces* (NAUMOV 1987; NAUMOV and NIKONENKO 1989). Second, the microorganisms identified as *S. cerevisiae* were not shown to have any unusual properties which might explain their presence in a human host.

This report describes the essential first step in the analysis of *S. cerevisiae* as a pathogen: the genetic characterization of clinical *S. cerevisiae* isolates. Stringent genetic tests are used to prove that the clinical isolates are *S. cerevisiae*. In addition, most of the clinical isolates are shown to be able to grow at 42°, a temperature which inhibits the growth of most other *S. cerevisiae* strains, a potential virulence trait. Finally, the ability to grow at 42° is shown to be a complex polygenic trait. [Portions of this work have been presented previously: Genetics Society of America Yeast Genetics and Molecular Biology Meeting, abstract 78A, 1991; 92nd Meeting of the American Society for Microbiology, abstract F64, 1992; Gordon Conference on Biological Regulatory Mechanisms, 1992; Gordon Conference on Fungal Metabolism, 1992.]

MATERIALS AND METHODS

Strains: Strains, with genotypes and origins, are shown in Table 1. One clinical isolate (generously provided by L. POTTER and C. PAPASIAN) will be referred to as YJM128 since TAWFIK *et al.* (1989) did not give the isolate a mnemonic. Other isolates are from the collection of the Infectious Disease Research Laboratory at the California Institute for Medical Research. All of these isolates are Gal^+ Mal^+ Suc^+ Spo^+ and have no auxotrophic requirements. The body site of isolation of the other isolates, as well as the results of experimental infections with both clinical and nonclinical isolates, are presented elsewhere (CLEMONS *et al.* 1994).

Techniques: Standard yeast genetic techniques were used (SHERMAN *et al.* 1974). Homothallic strains were sporulated to generate the mating-competent haploid spores used to mate with other strains or to select mutants. All incubations were done at 30° unless stated otherwise. Growth at different temperatures was tested by streaking cultures of cells (from a YEPD plate grown overnight at 30°) with a sterile toothpick onto a fresh YEPD plate in such a way that single cells would be deposited. Up to six strains (always including a positive control) were tested per plate. Plates were never stacked when temperatures ≥37° were being tested. Relative growth was determined by the size of colonies (if any). This method for testing growth at different temperatures was used, rather than liquid growth curves, because of the large number of strains and segregants to be tested and because of considerable variation in the degree of clumpiness and/or flocculence which causes gross errors in optical density measurements. A similar method for determining the ability to grow at different temperatures has been used to characterize and classify yeasts (BARNETT *et al.* 1990). Anaerobic incubation to score fermentation markers was done as described previously (MCCUSKER and HABER 1988).

TABLE 1

Yeast strains

Strain	Origin	Genotype
YJM128	Clinical isolate	
YJM145	Segregant of YJM128	*HO*
YJM155	YJM145	*HO lys2*
YJM222	Clinical isolate	
YJM345	Segregant of YJM222	*HO*
YJM413	Segregant of YJM345	*HO*
YJM417	YJM413	*HO lys2*
YJM273	Clinical isolate	
YJM280	Segregant of YJM273	*HO*
YJM377	YJM280	*HO lys2*
YJM308	Clinical isolate	
YJM319	Segregant of YJM308	
YJM425	Segregant of YJM319	
YJM426	Segregant of YJM425	
YJM427	Segregant of YJM426	
YJM428	Segregant of YJM427	*HO*
YJM429	YJM428	*HO lys2*
YJM309	Clinical isolate	
YJM320	Segregant of YJM309	*HO*
YJM378	YJM320	*HO lys2*
YJM310	Clinical isolate	
YJM326	Segregant of YJM310	*HO*
YJM376	YJM326	*HO lys2*
YJM311	Clinical isolate	
YJM339	Segregant of YJM311	*HO*
YJM379	YJM339	*HO lys2*
YJM312	Clinical isolate	
YJM352	Segregant of YJM312	
YJM419	Segregant of YJM352	
YJM421	Segregant of YJM419	*HO*
YJM424	YJM421	*HO lys2*
YJM25	Laboratory, S288c	α *lys2 gal2 SUC2 CUP1*
YJM27	Laboratory, S288c	α *lys5 gal2 SUC2 CUP1*
YJM28	Laboratory, S288c	**a** *lys5 gal2 SUC2 CUP1*
YJM237	YJM25/YJM28	α/**a** *lys2/+ +/lys5*
YJM400	Laboratory, S288c	**a** *ade2-101 his3Δ200 leu2Δ1 trp1Δ1 ura3-52 can1*
YJM401	Laboratory, S288c	α *ade2-101 his3Δ200 leu2Δ1 trp1Δ1 ura3-52 can1*
Y55	Laboratory, Y55	*HO*
Y55 *lys2*	Laboratory, Y55	*HO lys2*
Y55 *lys5*	Laboratory, Y55	*HO lys5*

The origin, derivation and genotypes of strains with related strains grouped together. Clinical *S. cerevisiae* isolates, one meiotic segregant from each isolate (which showed the best growth at high temperatures) and spontaneous *lys2* derivatives of each of these segregants are shown. *HO* strains appear to be diploid and euploid resulting from the self-diploidization of haploid spores. In cases where there is no genotype listed the ploidy and genotype is uncertain. Laboratory *S. cerevisiae* strains are shown with their genetic backgrounds, *i.e.*, S288c (the most commonly used laboratory genetic background) and Y55. Y55 is a homothallic, wild isolate (which has never been crossed with other laboratory strains) that retains the standard *S. cerevisiae* map order and distances (McCusker and Haber 1988) and has diverged significantly from the S288c genome at the molecular level (Nelson *et al.* 1993).

Media: YEPD (Sherman *et al.* 1974) was used for most manipulations. Fermentation phenotypes used similar media with a filter sterilized solution of the relevant sugar substituted for dextrose and added after autoclaving to a final concentration of 2%. Sporulation medium and defined media for scoring auxotrophy and canavanine resistance has been described previously (Sherman *et al.* 1974). α-Aminoadipate medium, used to select *lys2* auxotrophs, has been described previously (Chattoo *et al.* 1979).

RESULTS

Genetic characterization of putative *S. cerevisiae* clinical isolates: Two of the clinical isolates exhibited good spore viability (YJM222 and 273; Table 2). The spore viability pattern of YJM128 suggested heterozygosity for one recessive lethal mutation, while that for YJM308 and 309 suggested heterozygosity for two recessive lethal mutations (data not shown). Some of the clinical isolates exhibited low spore viability (YJM310, 311, 312; Table 2) for which there is no simple explanation. The presence of one or two recessive lethal mutations can be excluded by the recovery of tetrads with three or four viable spores. Similarly, a single chromosomal rearrangement can be excluded by the spore viability pattern (data not shown). Aneuploidy, perhaps in combination with a recessive lethal mutation or chromosomal rearrangement, may be responsible for the poor spore viability.

Segregants were tested for fermentation phenotypes, auxotrophy and sporulation. Most clinical isolates appeared to be heterozygous at one or more sugar fermentation loci and/or for auxotrophic mutations (Table 2). Most, if not all, of the segregants from all of the clinical isolates were capable of sporulation which suggested homothallism. Sporulation efficiency varied considerably among the segregants of some of the clinical isolates, suggesting heterozygosity for mutations which affect sporulation (data not shown). Considerable variation was also observed in the ability to grow at high temperatures (see below) as well as in colony size and morphology, clumpiness and/or flocculence and ability to replica plate (data not shown). These results show that the clinical isolates are diploid (or aneuploid) and that most are heterozygous at multiple loci which makes the isolates unsuitable for most studies. However, these characteristics are absent in segregants of the clinical isolates.

Isolation of segregants of clinical isolates for genetic analysis: More detailed analysis required the isolation of meiotic segregants of the clinical isolates which, when sporulated, would generate isogenic, mating-competent haploid spores. The following criteria were used to choose segregants for further analysis. First, the segregants, which are homothallic, must have a reasonably high sporulation efficiency since haploid spores must be used for performing crosses and mutant isolation. Second, the segregants should, like the parent, grow well at high temperatures (see below). Segregants meeting these criteria were sporulated and dissected to determine if they contained any heterozygosities that could arise if the parental clinical isolate strains were aneuploid.

In five cases (YJM128, 273, 309, 310, 311), segregants were found that met the above criteria (YJM145, 280, 320, 326 and 339, respectively) which, when sporulated

TABLE 2

Clinical isolates are heterozygous at multiple loci

Strain	GAL	MAL	SUC	Auxotroph	Recessive lethal[a]	Spore viability (%)
YJM128[b]	Yes (1)	Yes (2)	Yes (2)	No	Yes (1)	41.1 (143)
YJM222	No	No	No	No	No	81.7 (156)
YJM273[c]	Yes (1)	Yes (1)	No	No	No	92.3 (52)
YJM308[d]	No	Yes (2)	No	Yes (1)	Yes (2)	18.1 (234)
YJM309	No	No	No	No	Yes (2)	28.4 (104)
YJM310[a]	No	Yes (1)	No	No	No	66.0 (78)
YJM311[a,e]	No	Yes (1)	No	Yes (1)	No	36.1 (264)
YJM312[a]	Yes	Yes	Yes	No	No	45.3 (117)

Heterozygosity of clinical *S. cerevisiae* isolates for auxotrophic mutations, recessive lethals and fermentation markers (GAL, galactose; MAL, maltose; SUC, sucrose). The estimated number of heterozygous loci for a phenotype (in parentheses) assumes that the loci are unlinked, that the clinical isolates are diploid (not aneuploid or tetraploid). Spore viability (and the number of tetrads dissected) is also shown.

[a] The presence and number of recessive lethal mutations can be deduced from the percentage and the pattern of viable spores. One and two recessive lethal mutations will result in ~50% and ~25% spore viability, respectively, with a maximum of two viable spores/tetrad, *i.e.*, YJM128, 308, 209.

[b] YJM128 is heterozygous at two SUC loci that allow growth on raffinose and homozygous at one SUC locus that does not allow growth on raffinose.

[c] YJM273 is heterozygous for a mutation which causes slow growth and poor replica plating. Segregants carrying this mutation were not examined. Only one of 48 segregants which grew well proved to be Mal^+ indicating tight linkage between the Mal^+ allele and the deleterious mutation.

[d] YJM308 is a *met* heterozygote. When 20 *met* segregants were tested for their ability to mate, 13 were *MATα* and seven were nonmating.

[e] YJM311 is an *aro* heterozygote.

and dissected, yielded segregants that were indistinguishable from one another. This implies that haploid homothallic spores self diploidized to form sporulation competent diploids. In three cases (YJM222, 308, 312), it was necessary to undergo two, five and three generations (Table 1), respectively, of sporulation and dissection before obtaining segregants which met the above criteria. YJM308 and 312 (Table 2) and segregants of YJM222, 308 and 312 (data not shown), exhibited poor spore viability when dissected. Selected segregants of these strains were sporulated and dissected from each generation until segregants were found which, when sporulated and dissected, exhibited good spore viability with all segregants appearing to be identical. With two exceptions, the segregants of the clinical isolates chosen for further analysis showed spore viability in the range of 80–100% (data not shown).

The poor spore viability of YJM280 (33.7%) and 339 (60.6%) was also observed when the four segregants from a tetrad from each of these two strains, as well as YJM377 and 379 (the *lys2* derivatives of YJM280 and 339, respectively) were sporulated and dissected (data not shown). Crosses of YJM400 with YJM377 and 379 show that the spore inviability of YJM377 and 379 is recessive and dominant, respectively (Table 3).

Genetic proof that the clinical isolates are *S. cerevisiae*: Haploid spores of YJM155, 417, 377, 429, 378, 376, 379 and 424 were successfully crossed with the laboratory *S. cerevisiae* strains YJM400 and 401 (Table 1). Mating efficiency of the spores with *MAT***a** and *MATα* strains was approximately equivalent as determined by complementation (data not shown). These hybrids were sporulated and asci were dissected. Two types of control crosses were also done with YJM400: a cross with a closely related strain (YJM25) and a cross with an unrelated lab strain (Y55 *lys2*). Although there was some variability in colony size and morphology (compared to the YJM25/YJM400 control cross), the spore viability of the experimental crosses was surprisingly good (Table 3). Since intraspecific crosses within the genus Saccharomyces produce high spore viability while interspecific crosses yield no viable spores (NAUMOV 1986 1987; NAUMOV and NIKONENKO 1987, 1989) these results show that the clinical isolates are *S. cerevisiae*.

A total of 100 tetrads (89 tetrads for YJM379/YJM400) were tested from each cross and Mendelian segregation was observed for nine markers: *MAT*, *HO*, *ade2*, *his3*, *leu2*, *lys2*, *trp1*, *ura3* and *can1*. Recombination was shown to occur in eight intervals in all crosses: *trp1-HO*, *CEN15-ade2*, and *CEN2-lys2* (large intervals which are unlinked indicating that recombination occurred freely; data not shown), and *CEN5-ura3*, *CEN3-leu2*, *ade2-his3*, *ura3-can1*, *leu2-MAT*. Segregation data and recombination distances for the latter five intervals are shown in Table 3. In some cases recombination distances were found to differ from the control cross although there was no consistent pattern to these differences, *i.e.*, recombination distances might be smaller in one interval and be larger in another interval in the same cross (Table 3). Similar differences in map distances have been observed in *S. cerevisiae* (MORTIMER and SCHILD 1980). Since homeologous chromosomes from *Saccharomyces bayanus* undergo little or no recombination with *S. cerevisiae* chromosomes (NILSON-TILLGREN *et al.* 1981, 1986; CASEY 1986; PEDERSEN 1986) these results also show that the clinical isolates are *S. cerevisiae*.

TABLE 3

Recombination distances and spore viability from crosses with YJM400

Strains YJM400 ×	Intervals					Spore viability (%)
	ade2-his3	*CEN3-leu2*	*leu2-MAT*	*CEN5-ura3*	*can1-ura3*	
YJM25	38.2 (27:1:65)	6.0 (43:45:12)	32.5 (35:0:65)	9.5 (38:43:19)	56.6 (32:9:58)	97.6 (156)
Y55 *lys2*	44.9 (35:5:59)	7.4 (48:33:14)	26.5 (75.27)	5.6 (36:51:11)	34.2 (41:2:55)	84.4 (208)
YJM155	73.2 (20:13:64)	9.1 (45:36:18)	28.7 (67:27)	9.6 (41:31:19)	42.3 (35:4:58)	87.4 (208)
YJM376	42.4 (35:4:60)	12.0 (37:39:24)	23.0 (77:23)	8.0 (43:41:16)	30.8 (43:1:55)	94.8 (182)
YJM377	49.0 (32:6:61)	3.0 (46:47:6)	22.7 (75:22)	8.6 (39:43:17)	49.5 (31:6:61)	92.0 (208)
YJM378	49.5 (26:5:66)	13.5 (35:38:27)	20.4 (82:21)	13.0 (37:37:26)	42.9 (29:3:67)	88.0 (182)
YJM379	57.9 (26:8:55)	7.9 (34:40:14)	32.6 (58:28)	7.9 (42:32:14)	41.9 (34:4:48)	62.2 (364)
YJM417	35.7 (43:3:52)	4.5 (40:50:9)	23.8 (80:25)	12.0 (33:43:24)	37.4 (50:5:44)	74.1 (286)
YJM424	53.1 (24:6:66)	4.5 (27:63:9)	29.1 (68:28)	3.5 (45:47:7)	46.9 (31:5:61)	86.3 (208)
YJM429	55.0 (25:7:67)	3.5 (45:48:7)	24.0 (73:23)	6.5 (39:48:13)	33.7 (36:1:58)	84.8 (351)

Recombination distances (in cM) for five intervals (segregation data shown in brackets; parental ditypes (P):nonparental ditypes (N):tetratypes (T), except for crosses which are heterozygous for *HO* where recombination between *leu2* and *MAT* is given as: number of *ho leu2 MAT***a** segregants: number of *ho leu2 MATα* segregants) is shown for crosses between YJM400 and the listed strains (Table 1). Distances in the *ade2-his3*, *can1-ura3* and *leu2-MAT* (for YJM25/YJM400) intervals were determined as described by PERKINS (1949). Distances in the *leu2-MAT* interval for *HO/ho* crosses were calculated as the percentage of *ho leu2 MATα* segregants/all *ho leu2* segregants. Recombination in the *CEN3-leu2* and *CEN5-ura3* intervals was determined by monitoring the segregation of *leu2* and *ura3* with *trp1*; the formula $((T/2)/(P + N + T)) \times 100$ was used to calculate these distances. Spore viability (and the number of tetrads dissected) is also shown.

High temperature growth of clinical *S. cerevisiae* isolates: The ability to grow at >37° should be important for virulence because higher temperatures are found in febrile patients, *e.g.*, YJM128 came from a patient with a fever of 40.1° (TAWFIK *et al.* 1989). Growth at 42° was tested to allow finer discrimination between the ability of different isolates to grow at supraoptimal temperatures. Most of the clinical isolates (YJM128, 273, 309, 310, 311, 312) grew at 42° while laboratory yeast strains show no significant growth under these conditions (Figure 1).

Analysis of segregants of the clinical isolates for growth at 42°: Segregants of YJM128, 273, 309, 310, 311, 312 (which grow at 42°; Figure 1) were tested for their ability to grow at 42°. Segregants of YJM222 and 308 (which do not grow at 42° (Figure 1) and grow poorly at 39°, data not shown) were tested for their ability to grow at 39°. The ability of segregants of the clinical isolates to grow at 42° (YJM128, 273, 309, 310, 311, 312) and 39° (YJM222, 308) varied considerably: phenotypes ranged from no growth, growth of a dense streak of cells but no growth of single colonies, and growth of single colonies. There was considerable variability in colony size, among those segregants for which growth of single colonies was observed, forming a gradient of colony size from microcolonies to colonies that were as large (or almost as large) as the parental strain.

In the case of YJM222 and 308, which grow at 39° but not 42°, segregants were found that showed somewhat better growth than their parent strains at 39° (data not shown; examples in Table 1). In the case of YJM128, 273, 309, 310, 311 and 312, which grow at 42°, segregants were found that grow as well (YJM311, 312) or almost as well (YJM128, 273, 309, 310) as the parent strains at 42° (examples in Table 1; Figure 1). The number of segregants which showed the maximum observed growth at 42° (YJM128, 273, 309, 310, 311, 312) and 39° (YJM222, 308), and the total number of segregants tested, is given in Table 4. There are two possible explanations for the failure, in four cases (YJM128, 273, 309, 310), to find segregants which grew as well as the parent clinical isolate. First, these strains may be heterozygous at a large number of loci, in which case not enough segregants were examined. Second, alleles at two loci which are important for growth at high temperatures may be tightly linked and in *trans*.

The fact that only a small fraction of the segregants of the clinical isolates were able to grow at high temperatures shows that the clinical isolates are heterozygous at a number of loci for alleles important for growth at high temperatures. However, conclusions about the number of alleles required for growth at 42° cannot be drawn from the examination of the clinical isolates because these strains could be homozygous at any number of loci for alleles (dominant or recessive) important for high temperature growth or heterozygous at any number of loci for recessive alleles that are deleterious at high temperatures. The determination of whether high temperature growth is dominant or recessive requires that strains which can grow at high temperatures be crossed with defined laboratory strains. In addition, the number of genes required for high temperature growth (and whether the interactions are additive or epistatic) must be defined relative to some standard strain(s), *i.e.*, laboratory *S. cerevisiae* strains.

Crosses with laboratory strains: growth at 42°: Spores of YJM155, 417, 377, 429, 378, 376, 379 and 424 (Table 1) were crossed with YJM27 and spores of Y55 *lys5*. The 16 diploids were tested for their ability to grow at 42° (Figure 1) and placed in three classes. Class A consists of crosses between strains that can grow at 42° and laboratory strain backgrounds, producing diploids that can grow at 42°; a subset of class A are crosses that show

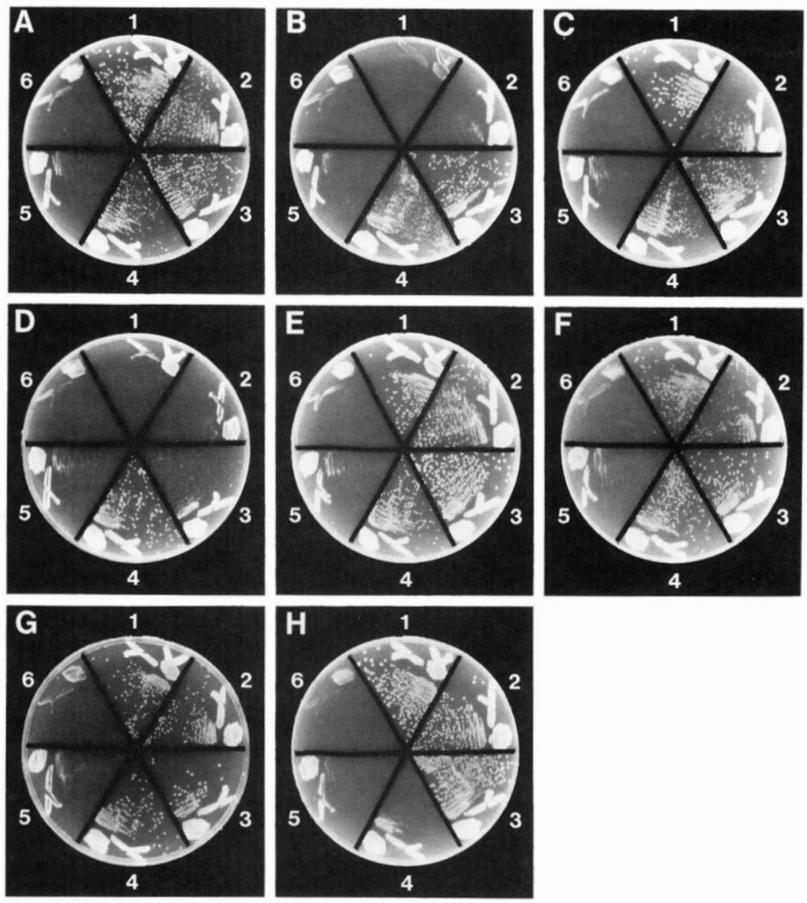

FIGURE 1.—Plates were incubated at 42° for 48 h. The identity of the prototrophic diploid strains on each plate (A through H) is given below: (1) a clinical isolate; (2) a segregant from that clinical isolate; (3) and (4) a cross between a *lys2* derivative of that segregant and YJM27 (isogenic with S288c) and Y55 *lys5* (isogenic with Y55), respectively; (5) YJM237 (isogenic with S288c); and (6) Y55. (A) 1, YJM128; 2, YJM145; 3, YJM155/YJM27; 4, YJM155/Y55 *lys5*. (B) 1, YJM222; 2, YJM413; 3, YJM417/YJM27; 4, YJM417/Y55 *lys5*. (C) 1, YJM273; 2, YJM280; 3, YJM377/YJM27; 4, YJM377/Y55 *lys5*. (D) 1, YJM308; 2, YJM428; 3, YJM429/YJM27; 4, YJM429/Y55 *lys5*. (E) 1, YJM309; 2, YJM320; 3, YJM378/YJM27; 4, YJM378/Y55 *lys5*. (F) 1, YJM310; 2, YJM326; 3, YJM376/YJM27; 4, YJM376/Y55 *lys5*. (G) 1, YJM311; 2, YJM339; 3, YJM379/YJM27; 4, YJM379/Y55 *lys5*. (H) 1, YJM312; 2, YJM421; 3, YJM424/YJM27; 4, YJM424/Y55 *lys5*.

improved growth at 42° (Table 4; Figure 1). Class B consists of crosses between strains that cannot grow at 42° that produce diploids able to grow at 42° (Table 4; Figure 1). YJM429/YJM27 may show marginally better growth at 42° and, if so, belongs in this class. Class C contains a single diploid that does not grow at 42° (YJM424/Y55 *lys5*; Figure 1) which is the product of a cross between a strain which does grow at 42° with a laboratory strain. These three classes show that alleles necessary for high temperature growth can be dominant, epistatic or recessive, respectively, depending upon the cross and suggests that different genetic strategies are used that will allow growth at 42°.

Segregation of the ability to grow at 42°: Hybrids between YJM155, 417, 377, 429, 378, 376, 379 and 424 (Table 1) and two unrelated laboratory strains (YJM27 and Y55 *lys5*) were sporulated and dissected. Fifty tetrads (200 segregants) from each of the 16 hybrids were examined for their ability to grow at 42°. The segregants from all of the hybrids showed an essentially continuous gradient of growth at 42° ranging from no colony formation, to microcolony formation and the formation of increasingly larger colonies, *i.e.*, 42° growth did not show Mendelian segregation in any of the crosses. This result indicates that the ability to grow at high temperatures is polygenic with additive effects of alleles at a number of loci. The number of segregants which showed the maximum observed growth in each of the 16 crosses, a qualitative measure of how well the segregants grew, and an estimate of the number of heterozygous loci involved in the ability to grow at 42° is given in Table 4.

The 16 diploids can be placed in three classes based on the maximum observed growth of the segregants relative to the parental diploid. Class D diploids produce some segregants that show growth equivalent to or slightly less than that of the parental diploid (Table 4). Class E diploids, the products of crosses between strains that fail to grow at 42°, do not grow at 42°, yet produce some segregants able to grow better than the parental diploid (Table 4). Class F consists of a single diploid (YJM424/Y55 *lys5*), in which the ability to grow at 42° is recessive, that produces segregants which grow significantly better than the parent. The class E diploids show that, in some cases, the alleles necessary for growth at 42° must be recessive and epistatic. These results also suggest that different genetic strategies are used to allow growth at 42°.

DISCUSSION

Clinical isolates are *S. cerevisiae*: Fungi are identified in clinical laboratories using tests (*e.g.*, sugar fermentation) that cannot distinguish "sibling" species in the genus *Saccharomyces* (*S. cerevisiae*, *S. paradoxus*, *S.*

TABLE 4

Growth at 42° and segregation of high temperature growth

Strains	42° growth (diploid)[a]		42° growth (segregants)[b]		No. of segregants/total (number of loci)[c]
YJM128	+++++		+++		7/235 (5)
YJM145	+++		NA		NA
YJM155/YJM27	++++	(A)	+++	(D)	6/200 (5)
YJM155/Y55 *lys5*	+++	(A)	+++	(D)	11/200 (4)
YJM222[d]	–		+		7/332 (5–6)
YJM413	+		NA		NA
YJM417/YJM27	+++++	(B)	+++	(D)	5/200 (5)
YJM417/Y55 *lys5*	++	(B)	+++	(E)	5/200 (5)
YJM273	+++++		+++		1/48 (5–6)
YJM280	+++		NA		NA
YJM377/YJM27	++++	(A)	++++	(D)	9/200 (4–5)
YJM377/Y55 *lys5*	+++	(A)	+++	(D)	26/200 (3)
YJM308[d]	+		+		9/134 (4)
YJM428	+		NA		NA
YJM429/YJM27	++	(B)	+++	(E)	5/200 (5)
YJM429/Y55 *lys5*	++++	(B)	+++	(D)	3/200 (6)
YJM309	+++++		++++		16/70 (2)
YJM320	++++		NA		NA
YJM378/YJM27	++++++	(A)	+++++	(D)	13/200 (4)
YJM378/Y55 *lys5*	++++	(A)	++++	(D)	21/200 (3)
YJM310	++++		+++		14/135 (3)
YJM326	+++		NA		NA
YJM376/YJM27	++++++	(A)	+++++	(D)	12/200 (4)
YJM376/Y55 *lys5*	+++++	(A)	+++++	(D)	18/200 (3–4)
YJM311	++++		++++		8/291 (5)
YJM339	++++		NA		NA
YJM379/YJM27	+++++	(A)	+++++	(D)	16/200 (3–4)
YJM379/Y55 *lys5*	++++	(A)	++++	(D)	29/200 (3)
YJM312	++++++		+++++++		35/212 (2–3)
YJM421	++++++		NA		NA
YJM424/YJM27	++++++	(A)	+++++	(D)	15/200 (3–4)
YJM424/Y55 *lys5*	+	(C)	++++	(F)	3/200 (6)
YJM237	+		NA		NA
Y55	+		NA		NA

Related strains are grouped together: the first strain in each group is a clinical isolate, the second is a segregant of that isolate and the third and fourth are crosses between a *lys2* derivative of that segregant and YJM27 and Y55 *lys5*, respectively. The 42° growth of diploids (42° growth-diploid) was determined by colony size (Figure 1) and is qualitatively represented by an open ended scale: (–) = no growth of the dense streak of cells: (+) = growth of the dense streak of cells but no colony formation; (++) = formation of microcolonies; ≥ (+++) = formation of increasingly larger colonies. The maximum observed 42° growth of segregants of these diploids (42° growth-segregants) is shown in the same way. The last column (no. of segregants/total) shows: the number of segregants with the maximum observed growth at 42°/total number of segregants tested. NA, not applicable, not heterozygous at loci involved in high temperature growth.

[a] Diploid classification based on the growth of the diploids relative to the parents. (A) Crosses between clinical isolate strain backgrounds which can grow at 42° and laboratory strain backgrounds which produce diploids able to grow at 42°. (B) Crosses between strains which cannot grow at 42° which produce diploids which can grow at 42°. (C) A diploid which cannot grow at 42°, the product of a cross between a strain can grow at 42° crossed with a laboratory strain.

[b] Diploid classification based on the growth of the segregants relative to the parent diploid. (D) Diploids produce some segregants which show growth equivalent or slightly less than that of the parental diploid. (E) Diploids and their parents do not grow at 42° yet produce some segregants which grow better than the parental diploid. (F) A diploid in which the ability to grow at 42° is recessive, produces segregants which grow significantly better than the parent.

[c] The number of heterozygous loci involved in the ability to grow at 42° (in brackets) is estimated by dividing the number of segregants examined for growth at 42° by the number of segregants showing the maximum observed growth at 42°. The quotient is approximately equal to 2^n where n is the estimated number of heterozygous loci.

[d] YJM222 and 308 do not grow at 42° and grow poorly at 39° but 7/332 and 9/134 segregants, respectively, exhibited better growth at 39°.

bayanus). Spore viability, Mendelian segregation of markers and recombination data show that chromosomes from clinical isolates have sufficient homology to chromosomes from laboratory *S. cerevisiae* strains that they are able to segregate and recombine normally. Such criteria differentiate the sibling species (NAUMOV 1986, 1987; NAUMOV and NIKONENKO 1987, 1989; NILSON-TILLGREN *et al.* 1981, 1986; CASEY 1986; PEDERSEN 1986). These results prove that the clinical isolates are *S. cerevisiae* and also that techniques and knowledge about *S. cerevisiae* are applicable.

Characterization and use of clinical *S. cerevisiae* isolates: Clinical isolates are only useful for phenotypic characterization (unusual traits which might account for their presence in human patients) and the generation of segregants which can be used for more detailed analy-

sis. This is because sporulation is necessary to generate haploid (expressing recessive mutations), mating-competent (for performing crosses) spores. Clinical isolate spores are not isogenic (due to multiple heterozygosities) and as a result every mutant isolated and every zygote formed by mating with another strain would be genetically different. Therefore, it is necessary to make segregants of the clinical isolates which can be used for mutant isolation and can be crossed since every haploid, mating-competent spore is isogenic. These segregants then can be manipulated in the same way as laboratory strains of *S. cerevisiae.*

How common is the ability to grow at high temperatures? There is no published survey of the maximum growth temperatures of a large number of *S. cerevisiae* strains. However, the National Collection of Yeast Cultures (NCYC) has determined the maximum growth temperature of 79 *S. cerevisiae* strains using a shaking gradient water bath and liquid defined medium (C. BOND, personal communication). The average of the maximum growth temperatures was 35° and only 5 of these 79 strains were able to grow between 39 and 40°. These 5 NCYC strains were obtained and tested for growth at 42° (as described in MATERIALS AND METHODS). Only one strain was found to grow well at this temperature while two other strains showed slight growth at 42°. This apparent increase in maximum growth temperature of some of the NCYC strains shows that the method (plates *vs.* liquid) and medium (YEPD *vs.* defined) used to determine growth at high temperatures can affect the results.

Eighteen other strains were tested for their ability to grow at 42°, including Y55 and YJM237 (Figure 1); ATCC 9763, four industrial strains from the Centraalbureau voor Schimmelcultures, one commercial yeast, Red Star, three recently collected wild isolates and four other *S. cerevisiae* strains from the University of California, Davis collection; and three additional *S. cerevisiae* strains from the NCYC (data not shown). None of these nonclinical *S. cerevisiae* strains was found to be able to grow well at 42°. It is clear that the ability to grow at very high temperatures is common in clinical strains and rare in nonclinical strains which suggests that the ability to grow at high temperatures may be a virulence trait in *S. cerevisiae.*

Why is the ability to grow at high temperatures important? The ability to grow at 37° has been shown to be important for fungal virulence (KWON-CHUNG 1982; MEDOFF *et al.* 1986). In addition, virulent *Candida albicans* can be distinguished from the closely related, avirulent *Candida stellatoidea* by its ability to grow at 42° (KAMIYAMA *et al.* 1989). Viewed in this light, the fact that most *S. cerevisiae* strains are unable to grow, or grow poorly, at ≥37° may partially account for the rarity of *S. cerevisiae* infections.

Some clinical *S. cerevisiae* isolates are capable of proliferation and persistence in immune competent, outbred mice (CLEMONS *et al.* 1992, 1994). Additional work has shown that there is a strong correlation between the ability to grow at 42° and the source of the isolates (clinical *vs.* nonclinical) and an even stronger correlation between the ability to grow at 42° and the ability to proliferate and persist (J. H. MCCUSKER, K. V. CLEMONS, D. A. STEVENS and R. W. DAVIS, manuscript in preparation). Therefore, the ability to grow at high temperatures may be viewed as a virulence trait in *S. cerevisiae.*

Ability to grow at 42° is a complex polygenic trait: The segregants of hybrids between YJM155, 417, 377, 429, 378, 376, 379 and 424 and laboratory strains showed a wide and virtually continuous range of growth at 42° with only a small fraction of the segregants being able to grow as well (or almost as well) as the parent at 42°. Since control crosses show Mendelian segregation for markers at nine loci, the simplest explanation for these results is that the ability to grow at 42° is polygenic with primarily, although probably not exclusively, additive effects. However, the wide variety of interactions seen in the crosses strongly suggests that there is more than one genetic strategy to extend the growth temperature range.

For example, in class A diploids (Table 4; Figure 1), the ability to grow at 42° is dominant. However, the ability to grow at 42° is not always dominant since the class C diploid cannot grow at 42° (Table 4; Figure 1). In addition, class B diploids (Table 4; Figure 1) show that the alleles required for growth at 42° can be dominant and have epistatic interactions. Finally, class E diploids produce segregants that show better growth than the parental diploids at 42° (Table 4) and must be heterozygous for recessive alleles important for growth at 42°. Taken together, these results strongly suggest that different genetic backgrounds contain different (but possibly overlapping) sets of alleles which govern maximum growth temperature.

Analysis of polygenic traits in *S. cerevisiae*: The interactions of polygenic trait loci could be defined in precise genetic and molecular terms if the individual loci could be cloned, sequenced, identified and characterized and then used to create a set of isogenic strains which would differ only at the relevant loci. However, the genetic complexity of high temperature growth suggests that standard yeast genetics techniques, which are geared toward working with single mutations with large phenotypic effects in isogenic strains, will be ineffective. For example, because of the minor effect of individual polygenic trait loci on phenotype, complementation testing and cloning are not feasible. In addition, mapping, using the standard techniques of yeast genetics, is impractical. First, the available markers are not sufficiently dense to map all of the polygenic trait loci simultaneously (which is very important). Second, the markers used to map the loci will affect the phenotype, *e.g.*, auxotrophic mutations affect growth.

Restriction fragment length polymorphisms (RFLPs) could be used to map the high temperature growth loci but this would require an enormous effort using standard methods because of the large genetic size of the yeast genome (~4500 cM; OLSON 1991). One alternative to RFLP mapping, genomic mismatch scanning (NELSON *et al.* 1993), has recently been developed which takes advantage of both the neutral nature and the high density of DNA polymorphisms between unrelated members of the same species and is far less laborious than the standard RFLP mapping methods. Once the high temperature growth loci have been mapped, they can be cloned, sequenced and identified and transformed into a single genetic background. Strains with defined genotypes can then be phenotypically tested to unambiguously determine both dominance and additivity *vs.* epistasis in precise genetic and molecular terms. *S. cerevisiae* can serve as a model for the analysis of polygenic traits such as quantitative traits.

***S. cerevisiae* as a model for the pathogenic fungi:** Clinical *S. cerevisiae* isolates have a number of properties (in addition to high temperature growth) that are unusual or not previously described for *S. cerevisiae*: elaborate pseudohyphae production, secretion of proteases and the ability to switch between morphologically and physiologically different cell types (J. H. MCCUSKER, K. V. CLEMONS, D. A. STEVENS and R. W. DAVIS, manuscript in preparation; J. H. MCCUSKER, unpublished observations). These characteristics, typically found in pathogenic fungi and thought to be virulence traits, may explain the presence of these clinical *S. cerevisiae* isolates in human hosts.

Experimental infections have shown that while nonclinical *S. cerevisiae* strains are rapidly eliminated *in vivo* in immune competent, outbred mice, some clinical *S. cerevisiae* strains are capable of both proliferation and long term persistence (Clemons *et al.* 1992, 1994) which are important aspects of pathogenicity. In addition, published case reports show that *S. cerevisiae* has been found to infect a variety of sterile body sites (AUCOTT *et al.* 1990; CIMOLAI *et al.* 1987; ENG *et al.* 1984; MANZELLA *et al.* 1989; NIELSEN *et al.* 1990; SETHI and MANDELL 1988; TAWFIK *et al.* 1989) which implies the ability to disseminate and invade as well as proliferate and persist *in vivo*. Taken together, this suggests that some strains of *S. cerevisiae* can be pathogenic under some circumstances.

Progress in the study of pathogenic fungi has been slow primarily because these organisms aregenetically intractable, *e.g.*, *C. albicans* is diploid and lacks a sexual cycle (*e.g.*, SCHERER and MAGEE 1990). The use of *S. cerevisiae* as a genetically manipulable model for pathogenic fungi, in the same sense that *S. cerevisiae* is used as a model eukaryote, will provide an important tool to aid in the understanding of fungal pathogenesis. In addition, a comparison of the clinical isolates of *S. cerevisiae* with other strains should provide insight into the evolution of a pathogen from a harmless saprophyte.

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