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Saccharomyces cerevisiae Virulence Phenotype As Determined with CD-1 Mice Is Associated with the Ability To Grow at 42°C and Form Pseudohyphae

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***Saccharomyces cerevisiae* isolates have been shown previously to exhibit a high degree of variation in their ability to proliferate and persist in CD-1 mice (K. V. Clemons, J. H. McCusker, R. W. Davis, and D. A. Stevens, J. Infect. Dis. 169:859–867, 1994). Isolate origin was not a firm predictor of virulence phenotype, since the virulence phenotypes of clinical and nonclinical isolates ranged from virulent to avirulent and from intermediate to avirulent, respectively. Therefore, it was important to determine if there was any association between putative virulence traits and virulence that might help explain the variation in virulence phenotypes. *S. cerevisiae* isolates spanning a range of virulence phenotypes in experimental infections were examined for putative virulence traits: the ability to grow at supraoptimal temperatures (42, 39, and 37°C), gelatin liquefaction, casein utilization, and pseudohyphal formation. Gelatin liquefaction appeared to be unrelated to pseudohyphal formation on casein or to virulence. Significant differences in the ability to grow at 39 and 42°C were observed when the virulent and intermediate classes were compared with the avirulent class. Less extreme but still significant differences in pseudohyphal formation were observed when the virulent and intermediate classes were compared with the avirulent class. Therefore, two virulence traits, similar to those identified in other pathogenic fungi, the ability to grow at elevated temperatures and pseudohyphal formation, have been identified in *S. cerevisiae*.**

Because of a long association with human food and drink, the yeast *Saccharomyces cerevisiae* has been considered to be a harmless saprophyte. However, there are a number of reports of infections due to *S. cerevisiae* (1, 5, 6, 9–11, 14, 16, 21, 25, 26, 28). These reports show that some isolates of *S. cerevisiae* can be clinically relevant. However, there has been no thorough characterization of the infecting microorganism in any of the reported cases. Although it is likely that some predisposing condition (e.g., immune deficiency) was an important factor in these cases, it is not known whether clinical isolates of *S. cerevisiae* themselves have unusual properties that are responsible for their presence in humans.

Previous work has shown that some clinical isolates were able to proliferate and persist in CD-1 mice, whereas nonclinical isolates could not (7). However, the clinical isolates showed a range of virulence phenotypes, forming a continuum of virulence to avirulence. In addition, nonclinical isolates, while showing a smaller range of virulence phenotypes, varied from an intermediate virulence phenotype to avirulent. Given these results, it became important to determine whether there was any association between virulence and potential virulence traits, which might help explain differences in virulence. This report shows that there are significant differences between virulent and avirulent isolates of *S. cerevisiae* with respect to the ability to grow at supraoptimal temperatures and pseudohyphal formation.

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MATERIALS AND METHODS

Yeast strains. The yeast strains characterized in this work are described in Table 1. Identification of clinical isolates as *S. cerevisiae* was determined by standard clinical microbiology tests (29). In addition, in most cases, a genetic test of species was also performed; i.e., mating-competent spores of segregants of clinical isolates were able to mate with *S. cerevisiae* laboratory strains. These hybrids were able to sporulate and, when dissected, yielded good spore viability and underwent normal meiotic recombination (17, 18).

Strains were placed into three groups with respect to origin as clinical, nonclinical, or laboratory constructs. Clinical isolates were recovered from different patients, at different times, and from a variety of geographic locations: YJM273, YJM454, and YJM222 in the San Francisco Bay area in 1989; YJM309, YJM310, YJM311, and YJM312 in the San Francisco Bay area in 1991; YJM440 and YJM455 in the San Francisco Bay area in 1992; YJM223 and YJM308 in southern California in 1990; YJM128 in Kansas City (28); YJM210 in Romania in 1930; YJM213 in Austria in 1935; and YJM436 in the United Kingdom. The body site of location of each of the clinical isolates has been described elsewhere (7). Nonclinical strains included industrial strains, recent wild isolates, and prototrophic diploids from two different laboratory strain genetic backgrounds (Y55 and S288c). Laboratory constructs were prototrophic strains constructed in the laboratory (Table 1). The derivation of one laboratory construct, YJM145, has been described previously (18). Crosses between strains with complementing auxotrophic mutations were made to generate the other laboratory constructs by using techniques described previously (18).

Virulent (Vir^+) strains have been defined as those strains

TABLE 1. *S. cerevisiae* virulence phenotypes and putative virulence traits

Strain	Original designation	Strain type[a]	Virulence[b]	Growth at[c]:			Pseudohyphal formation at[c]:		Gelatin liquefaction
				37°C	39°C	42°C	30°C	37°C	
YJM128[d,e,f]		C	+	+++	++	++	++	++	++
YJM145[f,g]		L	+/−	++	+	+	+	++	++
YJM210[h]	CBS 1227	C	+/−	+++	+	+	++	++	−
YJM213[h]	CBS 1464	C	+/−	+/−	+/−	−	++	++	−
YJM222[e,f,i,j]	89-156	C	−	++	+/−	−	+	−	++
YJM223[j]	90-79	C	+/−	+++	+	−	+	−	−
YJM224[k]	ATCC 9763	N	−	+++	++	−	+	++	+
YJM237[f,l]		N	−	+	+	+/−	+	−	−
YJM263	Fleischmanns	N	+/−	+++	++	+	+	++	+
YJM264	Red Star	N	−	−	−	−	−	−	−
YJM273[e,f,j]	VMC 132B	C	+	++	++	++	++	++	+
YJM274[m]		L	+	++	++	++	++	+	−
YJM276[m]		L	+	++	++	+	+	+	++
YJM277[n]		L	+/−	++	++	+	−	−	++
YJM308[e,f,j]	90-59	C	−	+	+/−	−	++	++	++
YJM309[e,f,j]	91-190	C	+	++	+	++	++	++	+/−
YJM310[e,f,j]	91-204	C	ND	++	++	++	+	+	+/−
YJM311[e,f,j]	91-212	C	+/−	++	++	++	++	++	−
YJM312[e,f,j]	91-213	C	+	+++	++	++	+	+	−
YJM332[o]	UCD 91-1	N	+/−	++	+/−	−	++	+	−
YJM334[o]	UCD 91-3	N	+/−	++	+/−	−	++	+	−
YJM336[o]	UCD 522	N	+/−	++	++	−	−	+	−
YJM337[o]	UCD 594	N	−	−	−	−	−	−	−
YJM338[o]	UCD 595	N	−	−	−	−	+	−	−
YJM431[p]		L	+/−	++	+	+	++	++	−
YJM436[q]	B70302(b)	C	+	++	++	++	+	+	−
YJM440[j]	92-123	C	+/−	++	++	++	+	++	+/−
YJM454[i,j]	89-156	C	+	++	+	+/−	+	+	+/−
YJM455	F4852	C	+/−	++	++	++	+	+	+/−
Y55[f,r]		N	−	++	+	−	+	+	+

[a] C, clinical; L, laboratory construct; N, nonclinical.
[b] +, Virulent; +/−, intermediate; −, avirulent; ND, not determined (data derived from reference 7).
[c] All strains were tested concurrently.
[d] Provided by L. M. Potter and C. J. Papasian (28).
[e] Genetic characterization is described elsewhere (18).
[f] 42°C growth phenotype has been shown previously (18); ability to grow at 42°C was determined again concurrently with all other strains (Fig. 3) shown to ensure that growth comparisons between strains were valid.
[g] Segregant of YJM128 (18).
[h] From the Centraalbureau voor Schimmelcultures, Delft, The Netherlands.
[i] YJM222 and YJM454 are subclones that differ in virulence (7).
[j] From the collection of D. A. Stevens and K. V. Clemons.
[k] From the American Type Culture Collection.
[l] A prototrophic diploid strain (18) that is isogenic with S288c (20), the most commonly used *S. cerevisiae* laboratory strain genetic background.
[m] YJM274 and YJM276 are the products of crosses of an auxotrophic derivative of YJM145 with S288c and Y55 genetic background laboratory strains, respectively.
[n] A diploid strain essentially congenic with YJM145 (17).
[o] University of California at Davis Department of Enology (UCD) culture collection, provided by L. Bisson. YJM332 and YJM334 (UCD 91-1 and 91-3, respectively) are the products of natural fermentations, i.e., wild or natural isolates. YJM336, YJM337, and YJM338 (UCD 522, 594, and 595, respectively) are industrial wine yeasts.
[p] A hybrid between Y55 and S288c laboratory background strains.
[q] Provided by F. Odds, Janssen Pharmaceutica, Beerse, Belgium.
[r] A wild isolate, thought to have been originally isolated by O. Winge, that has been in the laboratory for decades.

able to proliferate in the brains of immunocompetent outbred mice and to persist in significant numbers for ≥28 days (7). Strains with intermediate virulence ($Vir^{+/-}$) showed little or no proliferation and less persistence than Vir^{+} strains. Avirulent (Vir^{-}) strains did not proliferate and showed the least ability to persist.

Media. YEPD (1% [wt/vol] yeast extract, 2% [wt/vol] Bacto Peptone, 2% [wt/vol] glucose, 2% [wt/vol] agar) was used for most manipulations. Gelatin liquefaction plates were made essentially as described previously (27): 100 g of gelatin (Sigma G-2500; ~300 Bloom), 10 g of yeast extract, 20 g of dextrose, and 950 ml of distilled water were placed in a 55°C water bath (until the gelatin had melted) prior to autoclaving. Plates containing casein (Sigma C-0376) as the sole nitrogen source were made as follows. Yeast nitrogen base without amino acids and $(NH_4)_2SO_4$ (1.7 g), 2 ml of 1 M HCl, and 20 g of dextrose

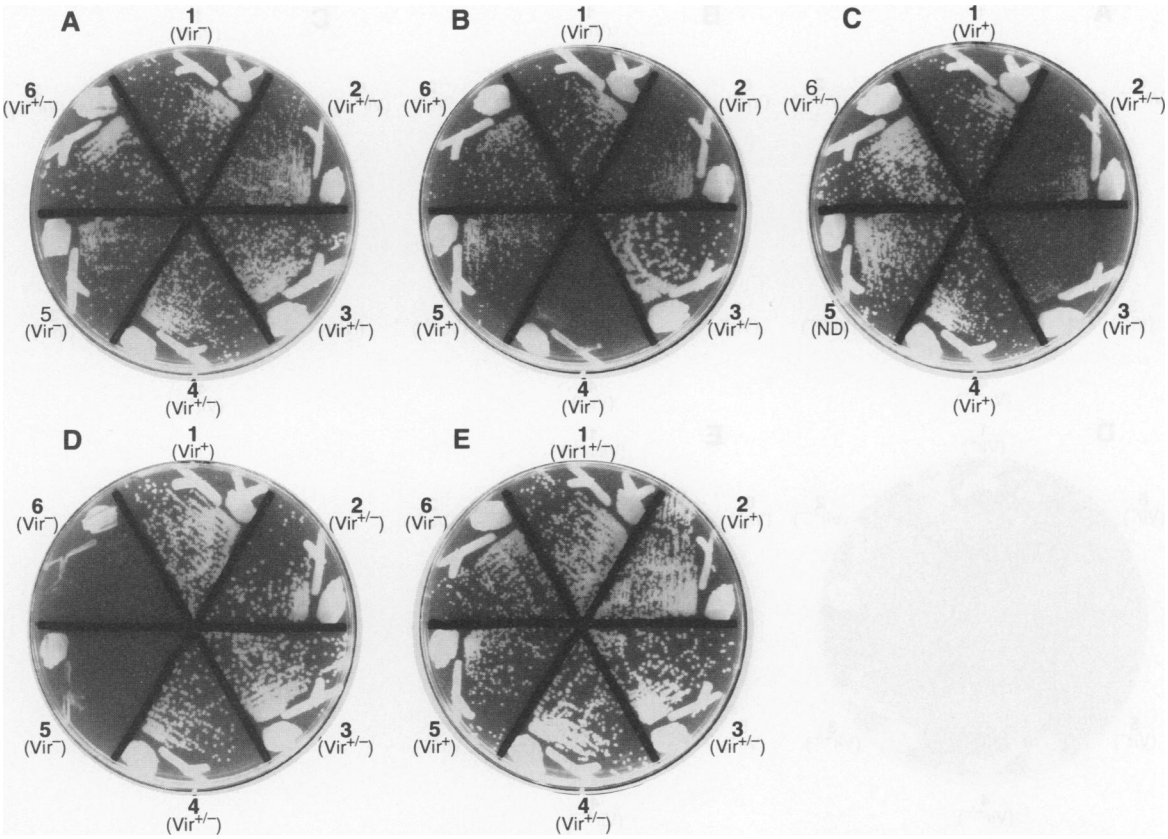

FIG. 1. Growth at 37°C after 26.5 h on a YEPD plate. (A) 1, YJM128; 2, YJM145; 3, YJM210; 4, YJM213; 5, YJM222; 6, YJM223. (B) 1, YJM224; 2, YJM237; 3, YJM263; 4, YJM264; 5, YJM273; 6, YJM274. (C) 1, YJM276; 2, YJM277; 3, YJM308; 4, YJM309; 5, YJM310; 6, YJM311. (D) 1, YJM312; 2, YJM332; 3, YJM334; 4, YJM336; 5, YJM337; 6, YJM338. (E) 1, YJM431; 2, YJM436; 3, YJM440; 4, YJM455; 5, YJM454; 6, Y55. Virulence phenotypes are also shown.

were dissolved in 100 ml of H_2O and filter sterilized. Casein (5 g) was dissolved in 400 ml of H_2O; ~800 mg of NaOH (eight pellets) was added to help dissolve the casein. The casein solution was adjusted to pH 10 with 1 M HCl and autoclaved. Agar (20 g) was autoclaved in 500 ml of H_2O. The three solutions were combined and poured into petri dishes.

Growth temperature. Yeast cells were grown at 30°C unless otherwise indicated. Growth at 37, 39, and 42°C was determined by streaking freshly grown cells onto plates and examining colony size (if any) after 1 to 2 days. Growth at temperatures >37°C was examined because such temperatures have been observed in febrile patients (e.g., 40.1°C [28]) and growth at 42°C allowed finer discrimination in relative growth. All strains were tested concurrently for growth at elevated temperatures to ensure that comparisons between strains were valid.

Protease secretion assays and pseudohyphal formation. Gelatin liquefaction was determined essentially as described previously (27) by transferring strains onto cold gelatin plates with sterile toothpicks and incubating the plates surface up at 25°C for up to 14 days. Liquefaction was scored qualitatively by the wet surface appearance of liquefying strains and by the degree to which strains caused dissolution of the gelatin and sank into the medium. The ability to utilize casein as the sole nitrogen source and pseudohyphal formation were determined by streaking cells onto the casein medium. Growth and pseudohyphal formation were determined after 14 days of incubation at both 30 and 37°C. All strains were tested concurrently to ensure that comparisons between strains were valid.

RESULTS

Growth at elevated temperatures. The ability to grow on YEPD plates at 37, 39, and 42°C was determined for all strains (Table 1; Fig. 1 to 3). The ability to grow at 37°C showed no significant association with virulence. However, growth at 39°C was significantly associated with virulence (Table 2). The ability to grow at 42°C was found almost exclusively in clinical strains (10 of 15 clinical strains were able to grow at 42°C, versus 1 of 10 nonclinical strains; $P = 0.007$, Fisher's exact test) and was tightly associated with virulence: 7 of 8 Vir^+ strains were able to grow at 42°C, versus 8 of 13 $Vir^{+/-}$ and 0 of 8 Vir^- strains (Table 2).

Gelatin liquefaction. The ability to liquefy gelatin (Gel^+ phenotype) was found in 5 of 15 clinical isolates and 3 of 10 nonclinical isolates (Table 1) and was not associated statistically with virulence. Because sporulating cultures of *S. cerevisiae* have been shown to secrete proteases (4) and liquefy gelatin (Beijerinck [1898] as cited in reference 4), Gel^+ strains were examined for spore formation on gelatin plates. Some, but not all, of the liquefying strains formed spores on this medium (data not shown).

Casein utilization and pseudohyphal formation. Strains

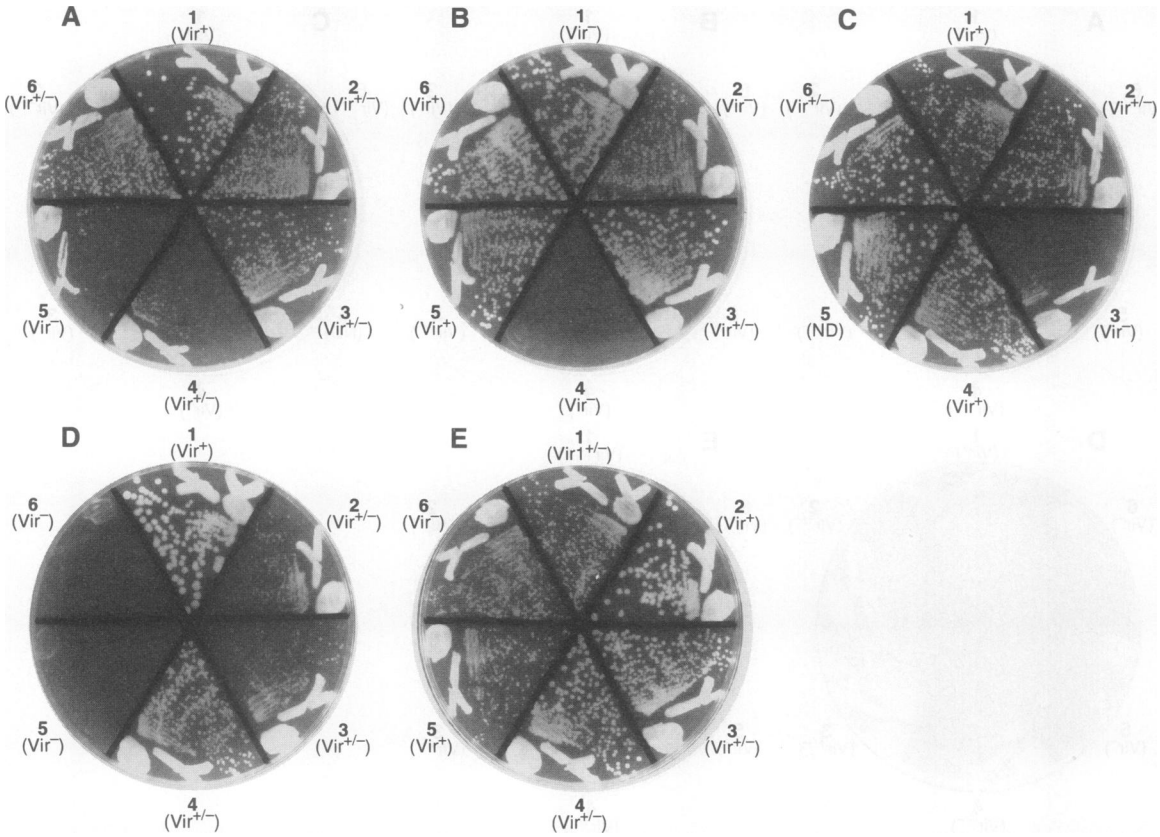

FIG. 2. Growth at 39°C after 43.5 h on a YEPD plate. Strains are as shown in Fig. 1.

were streaked (to deposit single cells) onto plates containing casein as the sole nitrogen source and incubated for up to 14 days at 30 and 37°C. The initial growth of all strains was quite rapid; colonies formed within 1 day at both temperatures. However, after this initial rapid growth, no strains grew well. The initial rapid growth may result from amino acids or small peptides, which would constitute a good nitrogen source, contaminating the casein since much less growth was seen when no nitrogen source was added (data not shown).

Microscopic observation of colonies grown on casein revealed that many of the strains formed pseudohyphae (Psh^+ phenotype) (Fig. 4). Pseudohyphal formation was seen more frequently, and tended to be more extensive (longer pseudohyphal chains and more pseudohyphal chains emanating from a colony), for clinical isolates but was not limited to these strains (13 of 15 clinical isolates formed pseudohyphae at 37°C, versus 6 of 10 nonclinical isolates; $P = 0.15$, Fisher's exact test). There was no difference in gelatin liquefaction between Psh^+ and Psh^- strains (8 of 23 Psh^+ strains [37°C] were Gel^+, versus 2 of 7 Psh^- strains; $P = 0.57$, Fisher's exact test). However, there was a significant association between pseudohyphal formation and virulence: 8 of 8 Vir^+ strains were Psh^+ versus 11 of 13 $Vir^{+/-}$ strains and 3 of 8 Vir^- strains (Table 2).

An examination of colonies growing on casein revealed features other than formation of pseudohyphae. In some cases, microcolonies were found below the surface of the agar (e.g., Fig. 4B, YJM273 and YJM440). In addition, while pseudohyphae were found below the surface of the agar, there were streams of cells on the surface around some of the colonies (e.g., Fig. 4A, YJM310, YJM336, YJM431, and YJM455). These consisted of a monolayer of elongated cells (not pseudohyphae) oriented in the same direction.

The association of phenotypic traits in subclones, a segregant, and crosses. Strains constructed or isolated in the laboratory and previously tested for virulence (7) were characterized phenotypically to determine the association of virulence with potential virulence traits: gelatin liquefaction, pseudohyphal formation at 37°C, and growth at 42°C (high-temperature growth [Htg]).

YJM222 (Vir^- Gel^{++} Psh^- Htg^-) and YJM454 (Vir^+ $Gel^{+/-}$ Psh^+ $Htg^{+/-}$) are phenotypically different subclones of one clinical isolate (Table 1). The virulent subclone showed less gelatin liquefaction, more pseudohyphal formation, and better growth at 42°C than the avirulent subclone.

The behavior of the Vir, Gel, Psh, and Htg phenotypes in crosses between different strains also was examined. Y55 (Vir^- Gel^+ Psh^+ Htg^-) and S288c (isogenic with YJM237; Vir^- Gel^- Psh^- $Htg^{+/-}$) genetic background strains were crossed to generate YJM431 ($Vir^{+/-}$ Gel^- Psh^{++} Htg^+). YJM431 did not liquefy gelatin (gelatin liquefaction was recessive in this case), showed more extensive pseudohyphal formation, grew better at 42°C, and resisted clearance to a greater extent than either of its parental genetic background strains (7).

YJM145 ($Vir^{+/-}$ Gel^{++} Psh^{++} Htg^+) is a segregant of YJM128 (Vir^+ Gel^{++} Psh^{++} Htg^{++}) (18). The $Vir^{+/-}$ segregant showed less growth at 42°C than its Vir^+ parent. An auxotrophic derivative of YJM145 ($Vir^{+/-}$ Gel^{++} Psh^+ Htg^+) was crossed with an S288c (isogenic with YJM237; Vir^- Gel^- Psh^- $Htg^{+/-}$) genetic background strain to generate YJM274 (Vir^+ Gel^- Psh^+ Htg^{++}). The ability to liquefy gelatin was

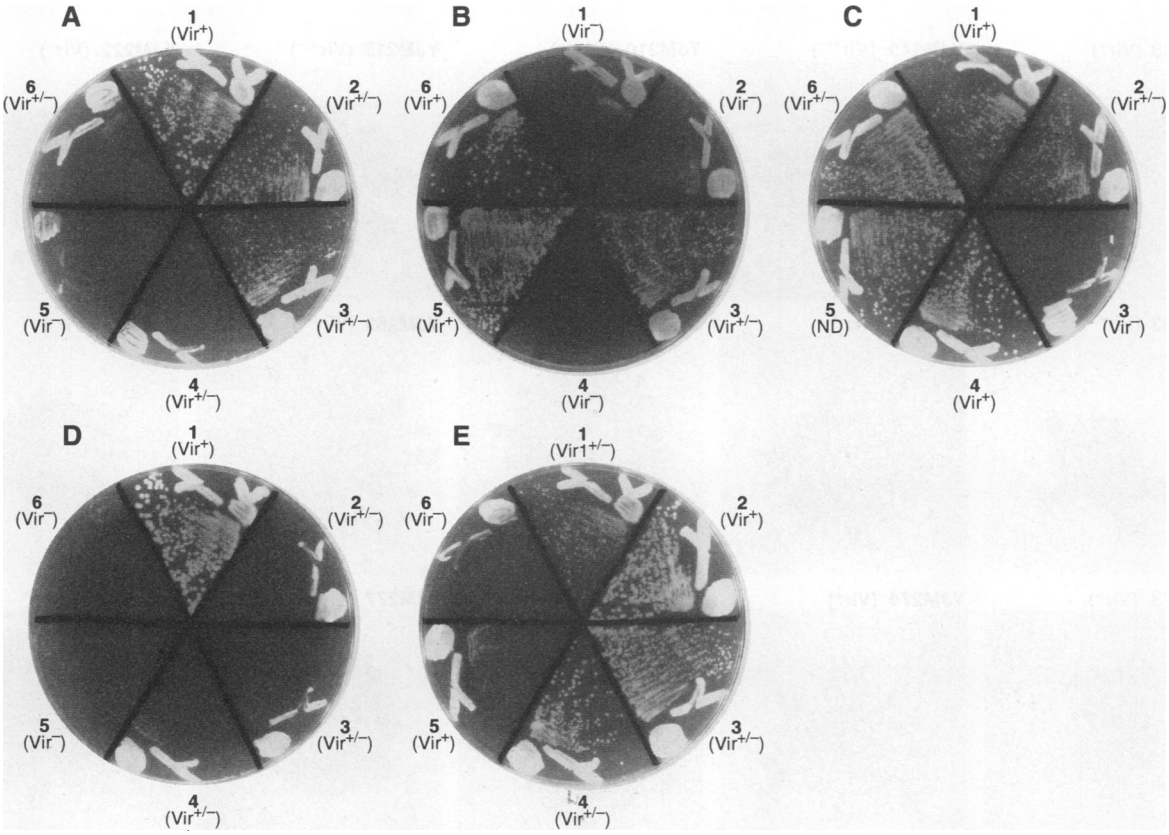

FIG. 3. Growth at 42°C after 48 h on a YEPD plate. Strains are as shown in Fig. 1.

recessive in YJM274, while the ability to form pseudohyphae was dominant. YJM274 was more virulent than either of its parents (7) and showed improved growth at 42°C. In addition, the same auxotrophic derivative of YJM145 was crossed with a Y55 (Vir^- Gel^+ Psh^+ Htg^-) genetic background strain to generate YJM276 (Vir^+ Gel^{++} Psh^+ Htg^+). YJM276 was able to grow at 42°C (the ability to grow at 42°C was dominant) and was more virulent (7) than either parental strain.

DISCUSSION

A number of published reports show that *S. cerevisiae* can be clinically relevant (1, 5, 6, 9–11, 14, 16, 21, 25, 26, 28). These reports indicate that infections due to *S. cerevisiae* are more common in immunodeficient individuals but are not limited to this class of patients. In addition, these reports show that *S. cerevisiae* can be recovered from normally sterile body sites. This is suggestive of the ability to proliferate, persist, disseminate, and invade, all of which are important characteristics for virulence. From these reports, it seems clear that some strains of *S. cerevisiae* can be pathogenic under some circumstances.

Clemons et al. (7) have shown that many of the clinical isolates described in this report differ significantly from more typical strains of *S. cerevisiae* (e.g., industrial strains) in the ability to proliferate and persist in immunocompetent mice. This report shows that strains defined as Vir^+ (7) differ from more typical Vir^- isolates of *S. cerevisiae* with respect to two putative virulence traits. The fact that these strains were independently isolated and appear to be unrelated, as judged by the results of classical biochemical and physiological tests (data not shown), restriction fragment length polymorphism analysis (data not shown), and, for many strains, genetic analysis (17, 18), lends additional weight to the association of putative virulence traits with virulence. An examination of subclones and crosses between different strains also gave results consistent with the association of virulence with pseudohyphal formation and the ability to grow at 42°C.

Logically, microorganisms unable to grow, or grow well, at temperatures ≥37°C should be avirulent or have reduced virulence with respect to the capacity to cause systemic infections. In fact, the ability to grow at 37°C has been shown to be a virulence trait for two pathogenic fungi (15, 19). In addition, two of the traits that distinguish virulent *Candida albicans* from the closely related avirulent *Candida stellatoidea* is that *C.*

TABLE 2. Fisher's exact test of statistical analyses of putative virulence traits with virulence

Trait tested[a]	*P*		
	Vir^+ vs Vir^-	$Vir^{+/-}$ vs Vir^-	Vir^+ vs $Vir^{+/-}$
Growth at:			
42°C	<0.001	0.006	0.221
39°C	0.013	0.090	0.215
37°C	0.100	0.133	0.619
PSH at 37°C	0.013	0.041	0.371

[a] Data shown in Table 1. Phenotype classes in Table 1 were simplified as follows to two classes to apply the Fisher's exact test, using the computer program STATS+ (Statsoft, Inc., Tulsa, Okla.): pseudohyphal formation (PSH) at 37°C, ++ and + were simplified to +; growth at 39 and 42°C, ++ and + were simplified to +, and +/− and − were simplified to −; growth at 37°C, +++, ++, and + were simplified to +, and +/− and − were simplified to −. A value of <0.05 was considered significant.

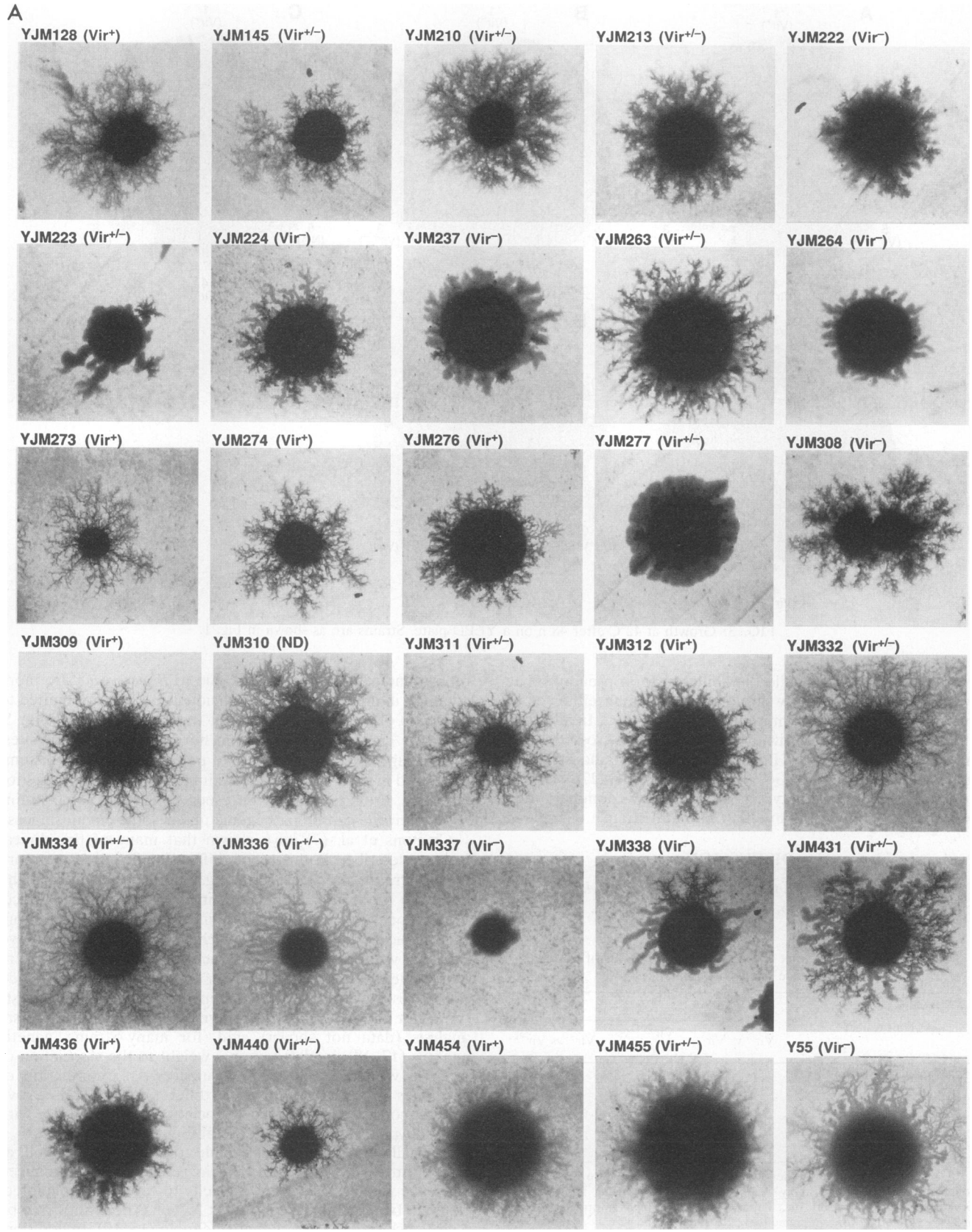

FIG. 4. Casein utilization and pseudohyphal formation at 30°C (A) and 37°C (B). Typical colonies are shown after 14 days on casein medium. All magnifications are equal. Strain numbers and virulence phenotypes are given above each frame.

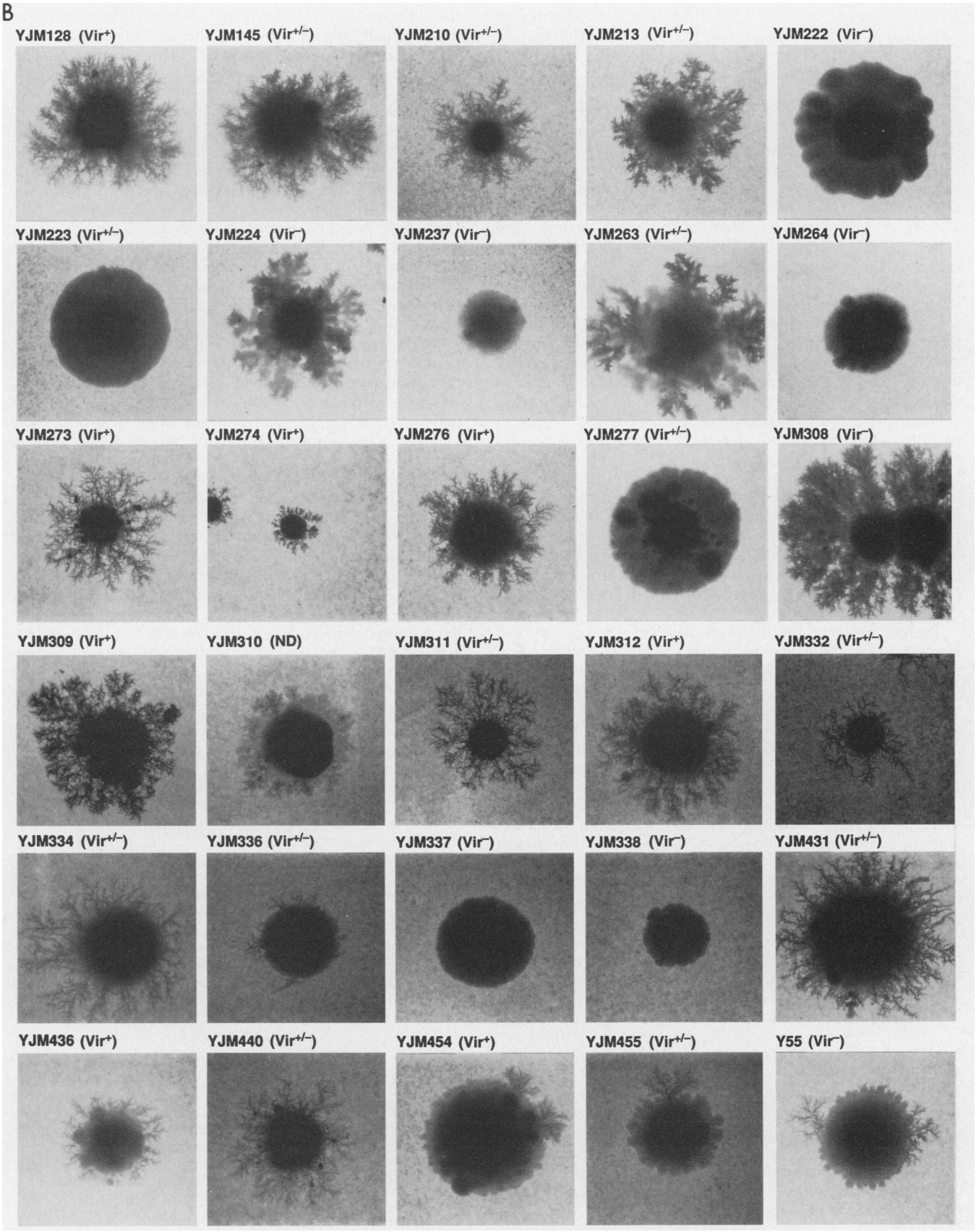

FIG. 4—*Continued.*

stellatoidea cannot grow at 42°C (13) or grow on minimal-glycerol medium at 37°C (23). The results in this report indicate that the ability to grow at 42°C on YEPD (but not the ability to grow on minimal-glycerol medium at 37°C [17]) was associated not only with isolate origin but also with virulence of *S. cerevisiae*.

If the ability to grow at high temperatures is atypical for *S. cerevisiae*, this might in part account for the rarity of infections due to *S. cerevisiae*. The average of the maximum growth temperatures observed for 79 *S. cerevisiae* strains (no clinical isolates) in the NCYC (National Collection of Yeast Cultures) collection was 35°C, using a liquid defined medium and a shaking gradient incubator. None of the NCYC strains were able to grow at temperatures greater than 40°C, and only 5 of the 79 strains grew between 39 and 40°C (3). These five NCYC strains were obtained and tested for growth at 42°C (as described in Materials and Methods). Only one strain was found to grow well at this temperature, while two of the others showed slight growth at 42°C. Also, nine additional nonclinical strains of *S. cerevisiae* have been tested and found to be unable to grow at 42°C (17). Thus, it is clear that the ability to grow at 42°C is not a common trait of *S. cerevisiae*.

Despite the strong association between the ability to grow at elevated temperatures and virulence, it must be emphasized that two pieces of evidence suggest that the ability to grow at high temperatures cannot be the sole determinant for virulence. First, three isolates that showed poor growth at 37°C on YEPD (YJM264, YJM337, and YJM338) all showed greater resistance to clearance from host tissues in experimental infections than did YJM237 (7), which can grow at 37°C. Second, YJM454, which was one of the most virulent isolates (7), showed much less growth at 42°C than did other much less virulent strains.

There is evidence that both protease secretion and hyphal formation are virulence traits in *C. albicans* (reviewed in reference 8). The strains of *S. cerevisiae* described in this report were tested for the ability to utilize exogenous casein as a sole nitrogen source as an assay for protease secretion. Although none of the strains tested grew well, many showed abundant pseudohyphal formation on this medium. Some strains formed microcolonies below the surface of the casein medium, suggesting the ability to switch to a nonpseudohyphal form, which may be better able to utilize exogenous casein as a nitrogen source. Pseudohyphal formation on casein medium was exhibited somewhat more frequently by clinical than nonclinical isolates, and the ability to form pseudohyphae on casein medium was associated with virulence.

Pseudohyphal formation was not as closely associated with clinical origin or virulence as was the ability to grow at 42°C. One reason for this may be that pseudohyphal formation, unlike the ability to grow at 42°C, is relatively common in *S. cerevisiae*, as can be seen from the data presented here. In addition, 210 of the NCYC strains of *S. cerevisiae* (no clinical isolates) have been tested for the ability to form pseudohyphae on potato dextrose agar and/or cornmeal agar. Of these, 99 formed pseudohyphae on these media (22). The ability to form pseudohyphae appears to be a common trait of *S. cerevisiae*. This may account for the lower association of pseudohyphal formation, compared with the ability to grow at 42°C, with virulence and strain origin.

The significant association of in vitro pseudohyphal formation with virulence is interesting since pseudohyphae have not been prominently observed to date in vivo (7). One explanation for this association may be that the process of penetrating agar is similar, but not identical, to the process of tissue invasion; i.e., characteristics other than pseudohyphal formation may be common to both types of invasion. Alternatively, the ability to form pseudohyphae on casein medium may be at least partially dependent upon the amount and/or type of extracellular protease(s) produced. In vivo expression of the same protease(s) by nonpseudohyphal cell types may supply *S. cerevisiae* with a nitrogen source or aid invasion.

There is evidence that the formation of pseudohyphae by *S. cerevisiae* is induced by starvation (12). However, the extensive formation of pseudohyphae on casein medium was not observed on other media previously used to determine pseudohyphal formation (potato dextrose agar and cornmeal agar [2, 12]) or on medium containing a low level of Casamino Acids or Bacto Tryptone (both derived from casein) as the sole nitrogen source (17). This finding suggests that utilization of contaminating amino acids and/or peptides followed by starvation may not be responsible for the abundant formation of pseudohyphae seen on the casein medium used in this study. Instead, the form of the nitrogen source (amino acids and peptides versus protein) may be important for the induction of extensive pseudohyphal formation. The fact that efficient pseudohyphal formation was seen on medium in which exogenous protein was the sole nitrogen source may be relevant to the association between pseudohyphal formation and virulence of *S. cerevisiae*.

Progress in the understanding of the pathogenic fungi has been slow, in part because the pathogenic fungi are, to different degrees, genetically intractable; e.g., *C. albicans* is diploid and lacks a sexual cycle (24). *S. cerevisiae*, by comparison, has been extensively studied and is easily manipulated. Thus, *S. cerevisiae* may serve as a model for the genetically intractable fungi in the same sense that it serves as a model eukaryote. The identification of traits or phenotypes associated with virulence is only one step in the analysis of virulence. Detailed genetic analysis will be required to elucidate the mechanisms of individual virulence traits (e.g., the ability to grow at 42°C), the relative importance of different virulence traits (e.g., the ability to grow at 42°C versus pseudohyphal formation), and the identity of other, less obvious, virulence determinants. In addition, it is possible that the use of a different host may uncover different virulence determinants. Finally, the genetic analysis of differences between virulent and avirulent strains will aid in the understanding of how a pathogen might evolve from a harmless saprophyte.

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