'Fusel' alcohols induce hyphal-like extensions and pseudohyphal formation in yeasts

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At a concentration of 0.5% (v/v), isoamyl alcohol induced the formation of hyphal-like extensions in haploid and diploid strains of *Saccharomyces cerevisiae* in liquid complex medium. These extensions, which develop via bud initiation and elongation, undergo DNA replication and nuclear division and appear similar in many respects to an aberrant form of the cell division cycle. However, in 0.25% (v/v) isoamyl alcohol, *S. cerevisiae* formed pseudohyphae. Other 'fusel' alcohols (which are the products of amino acid catabolism) also induced hyphal-like extensions in this yeast, with *n*-amyl alcohol being as equally effective as isoamyl alcohol. Isoamyl alcohol induced the formation of pseudohyphae in two species of *Candida* and both hyphal-like extensions and pseudohyphae in *Brettanomyces anomalus*, suggesting a close relationship or a common basis to the development of the two morphologies.

Keywords: *Saccharomyces cerevisiae*, yeasts, pseudohyphae, isoamyl alcohol, 'fusel' alcohols

INTRODUCTION

The yeast *Saccharomyces cerevisiae* is a favourite organism for investigating many aspects of cell biology, including the cell cycle, signal transduction, development and differentiation. In the last 4 years, the development of pseudohyphae in this yeast has received considerable attention (Gimeno *et al.*, 1992; Wright *et al.*, 1993; Blacketer *et al.*, 1993; Kron *et al.*, 1994; Roberts & Fink, 1994; Kuriyama & Slaughter, 1995). The predominant view is that starvation for nitrogen is the signal for the switch from the yeast to a filamentous form (Kron *et al.*, 1994), although it has also been shown that pseudohyphal growth is strictly oxygen-dependent (Wright *et al.*, 1993) and that limitation of oxygen during continuous cultivation can result in the formation of pseudohyphae (Kuriyama & Slaughter, 1995).

I recently observed that culturing *S. cerevisiae* in a liquid minimal medium in which ethanol is the carbon source and leucine the nitrogen source results in the formation of what were called large pseudohyphae (Dickinson, 1994), but which are probably better termed 'hyphal-like extensions' (a phrase used recently by Black *et al.*, 1995). For clarity, pseudohyphae are defined here as chains of regular-shaped, elongated cells in which polar budding predominates. A hyphal-like extension is taken to mean a structure originating from a bud which has undergone abnormal length extension so as to exceed the length of

the mother cell from which it arose, but without the isotropic swelling characteristic of the normal yeast cell cycle. In furtherance of my previous observation (Dickinson, 1994), this paper describes the sequence of experiments which revealed that 'fusel' alcohols (which are the natural metabolic products of the catabolism of certain amino acids) trigger the formation of hyphal-like extensions and pseudohyphae in both *S. cerevisiae* and *Brettanomyces anomalus* and pseudohyphae in two species of *Candida*. This suggests a close relationship or a common basis to the development of the two morphologies.

METHODS

Organisms used. *Saccharomyces cerevisiae* prototrophic wild-type strain IWD72 (*MAT α*) (Dickinson & Dawes, 1992) was used for most purposes. W303-1A (*MAT α ade2-1 ura3-52 leu2-3,2-112 trp1-1 his3-11,3-15*) came from R. Rothstein, College of Physicians and Surgeons, Columbia University, New York, USA. Respiratory-deficient 'petite' derivatives of IWD72 and W303-1A which lack mitochondrial DNA were isolated by treatment with ethidium bromide on solid YEPD medium (1%, w/v, yeast extract; 2%, w/v, bacteriological peptone; 2%, w/v, glucose; 0.01% adenine; and 0.01% uracil). A. G. Hinnebusch (NIH, Bethesda, MD, USA) provided the haploid strain H2388 (*MAT α gcn4::LEU2 ura3-52 leu2-3,2-112 trp1- Δ 63*) and the congeneric wild-type H1486 (*MAT α ura3-52 leu2-3,2-112 his1-29 [HIS4-lacZ ura3-52]*). Σ 1278b (*MAT α*) was obtained from J.-M. Wiame, CERIA, Brussels, Belgium. The congeneric series of strains L5626 (*MAT α /MAT α ura3-52/ura3-52 leu2/leu2 ste7::LEU2/ste7::LEU2*), L5625 (*MAT α /MAT α ura3-52/ura3-52 leu2/leu2 ste11::LEU2/ste11::LEU2*), L5627 (*MAT α /MAT α ura3-52/ura3-52 leu2/leu2 ste12::LEU2/*

Abbreviation: DAPI, 4',6-diamidino-2-phenylindole.

ste12::LEU2) and L5624 (*MATa/MAT α ura3-52/ura3-52 leu2/leu2 ste20::LEU2/ste20::LEU2*) was developed in G. R. Fink's laboratory (MIT, Cambridge, MA, USA) and was supplied by N. A. R. Gow, University of Aberdeen, UK. *Candida boidinii* CBS 5777 originated from the Centraalbureau voor Schimmelcultures, Delft, The Netherlands. *Candida albicans* wild-type strain 3153 was from A. J. P. Brown, University of Aberdeen, UK. *Brettanomyces anomalus* NCYC 449 was obtained from The National Collection of Yeast Cultures, Norwich, UK.

Growth conditions. All of the organisms were cultured at 30 °C in complex liquid medium (YEPD) as described previously (Dickinson & Dawes, 1992). Starter cultures were grown in YEPD to an OD₆₀₀ of 10. These cells were then used to inoculate fresh cultures containing either ethanol minimal medium with leucine (EML: 2%, v/v, ethanol; 2%, w/v, leucine; and 0.16%, w/v, Difco Yeast Nitrogen Base without amino acids and ammonium sulfate) (Dickinson, 1994) or YEPD containing isoamyl alcohol to an OD₆₀₀ of 0.1. Metabolic products were determined in culture supernatants by GC using 80/100 Carbowax C/0.2% Carbowax 1500 with an oven temperature of 135 °C and nitrogen as carrier at 20 ml min⁻¹. Staining with 4',6-diamidino-2-phenylindole (DAPI) was done as described by Sherman *et al.* (1986).

RESULTS AND DISCUSSION

The metabolites of leucine metabolism trigger the formation of hyphal-like extensions

The GCN4 protein is well-known for its central role in the general control of nitrogen metabolism (Hinnebusch, 1992). When the *gcn4* mutant H2388 was grown in YEPD and then transferred to EML, it formed hyphal-like extensions (Fig. 1a). This occurred slightly more slowly than in the wild-type strains used previously (Dickinson, 1994); nevertheless, 288 h after transfer to EML, typically 27–28% of the culture had produced hyphal-like extensions. Since the congenic wild-type strain H1486 (*GCN4*) behaved similarly to H2388, it seems reasonable to assume that the slower response was due to the general genetic background. Hence, GCN4 is not required for the

formation of hyphal-like extensions and one may conclude that the cells are not doing this in response to the level of uncharged tRNA's. Glutamate occupies a central position between nitrogen catabolism and anabolism (Cooper, 1982). However, the addition of up to 2% (w/v) glutamate did not prevent the formation of hyphal-like extensions (data not shown), which suggests that the formation of these structures is not a response to nitrogen starvation.

When a high concentration of leucine is given to *S. cerevisiae*, starvation for isoleucine and valine can result because leucine feedback inhibits the *LEU4* gene product (α -isopropylmalate synthase), thus reducing the synthesis of α -isopropylmalate. α -Isopropylmalate is believed to act as co-inducer with the LEU3 protein of the biosynthetic pathways (Hinnebusch, 1992). To examine whether hyphal-like extensions were formed in EML due to starvation for isoleucine and/or valine, these amino acids were added to EML (20 μ g ml⁻¹ final concentration). This resulted in a much greater proportion of the culture forming hyphal-like extensions (100% by 146 h) than when isoleucine and valine were omitted. Clearly, specific starvation for isoleucine and valine could not be the cause because addition of these amino acids accelerated and enhanced the morphological change. Hence, neither general nitrogen starvation nor starvation for specific amino acids seems to be required for the formation of hyphal-like extensions.

A possible explanation was that one or more intermediates or products of leucine metabolism was responsible for the formation of hyphal-like extensions in liquid EML medium. In *S. cerevisiae*, the first two steps of branched-chain amino acid catabolism are similar to those in other eukaryotes in that they involve first an aminotransferase and then 2-oxoacid dehydrogenase (Dickinson & Norte, 1993; Dickinson & Dawes, 1992). However, in yeast, major products of branched-chain amino acid catabolism are the so-called 'fusel' alcohols (Webb & Ingraham,

Fig. 1. Formation of hyphal-like extensions by *S. cerevisiae*. (a) *gcn4* mutant H2388 after being cultured in EML medium; (b) wild-type haploid IWD72 8 h after transfer to YEPD medium containing 0.5% (v/v) isoamyl alcohol; (c) a typical field of IWD72 24 h after transfer to YEPD containing 0.5% (v/v) isoamyl alcohol. Bar, 50 μ m.

Table 1. Metabolic products resulting from culturing prototrophic haploid IWD72 in ethanol minimal media with different nitrogen sources

The results (expressed in $\mu\text{g ml}^{-1}$) are the means of triplicate determinations; standard errors were $\leq 6.7\%$.

Compound	Nitrogen source		
	Valine	Leucine	Isoleucine
Acetaldehyde	3	71	62
Active amyl alcohol	22	23	531
Ethyl acetate	< 1	< 1	< 1
<i>n</i> -Propanol	2	1	5
Isobutanol	475	5	23
Isoamyl acetate	< 1	< 1	< 1
Isoamyl alcohol	29	808	25

1963). [The German word '*fusel*', perhaps best translated to the colloquial English '*rotgut*', is used to refer to the mixture of compounds present in the '*fusel oil*' which remains after most of the ethanol has been removed from any yeast fermentation. The chief constituents of fusel oil are isoamyl alcohol (3-methylbutan-1-ol), active amyl alcohol (2-methylbutan-1-ol), *n*-amyl alcohol (pentan-1-ol), isobutyl alcohol (2-methylpropan-1-ol), *n*-butanol (butan-1-ol), *n*-propanol (propan-1-ol), various esters of these alcohols and lesser quantities of *n*-hexanol (hexan-1-ol) and 2-phenylethanol.] The prototrophic wild-type haploid IWD72 was raised in liquid YEPD and then transferred to ethanol (2%, v/v) minimal medium with 2% (w/v) leucine (EML), 2% (w/v) isoleucine or 2% (w/v) valine as the sole source of nitrogen as described previously (Dickinson, 1994). As expected, this resulted in the formation of hyphal-like extensions in EML (25%) but not in the other two media. Three hundred and ten hours after transfer to ethanol minimal media, the cells were harvested by centrifugation and the culture supernatants were analysed by GC. When leucine was the sole source of nitrogen, the yeast produced large amounts of isoamyl alcohol; with isoleucine active amyl alcohol was produced and when valine was the nitrogen source isobutanol was the major alcohol detected (Table 1). These results were expected (Webb & Ingraham, 1963) and prompted an examination of whether hyphal-like extensions are formed in YEPD medium to which isoamyl alcohol has been added.

The addition of isoamyl alcohol to liquid YEPD resulted in rapid formation of hyphal-like extensions in a dose-dependent manner (Table 2). Hyphal-like extensions were observed in significant proportions by 24 h; this is far quicker than in EML medium, where they are never observed in wild-type strains before 80 h. The lowest concentration of isoamyl alcohol added (0.08%, v/v) was chosen to mimic the concentration detected in EML medium (Table 1). Isobutyl alcohol was also added at 0.0005% (v/v) in the presence of 0.08% isoamyl alcohol to approximate the concentration of this alcohol detected

Table 2. Effect of isoamyl alcohol on yeast morphology in liquid YEPD medium

Prototrophic haploid IWD72 was grown in YEPD to OD_{600} 10.0. Aliquots were removed and inoculated into fresh 100 ml batches of YEPD medium containing different concentrations of isoamyl alcohol to give an OD_{600} of 0.1. The formation of hyphal-like extensions was quantified by counting at least 400 cells or extension-bearing structures in each sample. The results show the percentage of hyphal-like extensions in the cultures and are the means of two separate experiments; standard errors were approximately 3%. ND, Not determined.

Time (h)	Concentration of isoamyl alcohol added (% v/v)			
	0.08	0.08 + 0.0005 % isobutanol	0.16	0.32
24	5	6	8.5	25.5
44	2	2	7.5	31.5
71	0.5	0.5	6.5	41.0
124	ND	ND	ND	25.0

Table 3. Effect of higher concentrations of isoamyl alcohol on yeast morphology

The strain and conditions were identical to those described in Table 2. The results show the percentage of hyphal-like extensions in the cultures and are the means of two separate experiments; standard errors were approximately 3%. ND, Not determined.

Time (h)	Isoamyl alcohol concentration (% v/v)			
	0.32	0.4	0.5	0.6
3.5	16.5	49.0	53.5	24.5
8	20.1	53.5	69.5	53.0
24	24.0	15.5	73.0	77.0
78	24.0	32.0	26.0	ND

after culture of yeast in EML (Table 1). It is not clear from the experiment reported in Table 2 whether isobutyl alcohol has any important effect on the formation of hyphal-like extensions (but see Table 4 below). After 24 h, the proportion of hyphal-like extensions decreased with time in the two lower concentrations of isoamyl alcohol. In 0.32% isoamyl alcohol, the proportion of hyphal-like extensions declined after about 70 h (Table 2). This suggests that *S. cerevisiae* has a mechanism to degrade this compound or somehow reverse its effects in rich medium. In control cultures lacking isoamyl alcohol, no hyphal-like extensions were observed at any time.

A further experiment examined the effect of increased concentrations of isoamyl alcohol in liquid YEPD. A concentration of 0.5% (v/v) isoamyl alcohol resulted in optimal production of hyphal-like extensions, with well over 50% of the culture showing this morphology after 3.5 h (Table 3). By 8 h, 69% had hyphal-like extensions in

Fig. 2. DAPI staining of haploid *S. cerevisiae* after 24 h in liquid YEPD medium containing 0.5% (v/v) isoamyl alcohol. Wild-type haploid IWD72 was stained with DAPI and observed under UV (a) and visible (b) light. A few details that can be seen clearly in (b) are apparently 'missing' in (a); this is due to the lower light levels under UV. Bar, 50 µm.

0.5% isoamyl alcohol and looked very like *C. albicans* when it switches from yeast to a hyphal form (Fig. 1b). By 24 h, well over 70% of cells had hyphal-like extensions, some of which were as long as 68 µm (Fig. 1c) in the presence of 0.5% isoamyl alcohol. After 100–120 h, many of these extensions reached lengths in excess of 100 µm. The proportion approached 80% (Table 3) when the isoamyl alcohol concentration was increased to 0.6%, but all of the structures were smaller than those formed in 0.5% and they looked generally less healthy. In 0.4% isoamyl alcohol, there appeared to be two waves of extension formation because there was a minimum at 24 h. This might be due to a degree of synchrony in the development of hyphal-like extensions coupled with the time-dependent recovery from isoamyl alcohol noted above.

The results reported were not unique to the one strain illustrated; similar results were obtained with a range of haploids of both α - and α -mating type from a variety of different genetic backgrounds. Nevertheless, there was a variation in performance between strains from different genetic backgrounds. In particular, the W303-1A background seemed especially unsuitable, with a consistently lower proportion of cells forming hyphal-like extensions. The genetic background of Σ 1278b is considered to be especially good for pseudohyphal formation upon nitrogen starvation (Gimeno *et al.*, 1992); however, it conferred no special advantage or disadvantage towards the ability to form hyphal-like extensions in the presence of 0.5% (v/v) isoamyl alcohol. 'Petites' completely lacking mitochondrial DNA are able to proliferate in YEPD but were unable to form hyphal-like extensions 24 h after exposure to isoamyl alcohol. However, such strains did form low proportions (1–3%) of hyphal-like

extensions after prolonged incubation, indicating that the cells do not need to be respiratorily competent in order to form these structures. Thus, it would seem that 'petites' were merely slower than wild-type ('grande') strains because of their generally reduced metabolic rate. Diploids formed hyphal-like extensions to even greater extents than haploids, typically yielding 97–100% hyphal-like extensions after 24 h in YEPD containing 0.5% (v/v) isoamyl alcohol. All strains tested also formed hyphal-like extensions 24 h after being streaked-out or spread onto solid YEPD containing isoamyl alcohol, although this occurred optimally at 0.6% (v/v) isoamyl alcohol rather than 0.5% (v/v) in liquid medium. On solid media, diploids starved for nitrogen formed pseudohyphae and haploids exhibited invasive filaments after 4 d on YEPD as described by Fink and co-workers (Gimeno *et al.*, 1992; Roberts & Fink, 1994). This confirms that the formation of hyphal-like extensions was not a peculiarity of the strains used.

An important question is 'Are the effects induced by isoamyl alcohol pathological or physiological?' This seems to depend upon the concentration of isoamyl alcohol used. On the one hand, the hyphal-like extensions induced in *S. cerevisiae* by 0.5% (v/v) isoamyl alcohol are reminiscent of an aberrant form of the cell division cycle; e.g. elongations are produced as a result of *cdc3*, *cdc4*, *cdc10*, *cdc11*, *cdc12*, *cdc34*, *cdc55*, *yak1 yak2* double mutants and overexpression of *AFR1*, *CLN1* or *GLC7* (Konopka *et al.*, 1995; Healy *et al.*, 1991; Zhang *et al.*, 1995; Black *et al.*, 1995; and references therein). This conclusion is supported by DAPI staining, which shows that the extensions contain several DAPI-staining bodies: on average, one in each individual 'bulb' of an extension (Fig. 2). Since DAPI stains mitochondrial DNA as well as

nuclear DNA in fixed cells, this can sometimes be a problem. However, although as noted above, 'petites' form much lower proportions of hyphal-like extensions and take longer to do it, similar staining was observed in the hyphal-like extensions formed by the related strain IWD72 p^- , which lacks mitochondrial DNA. In contrast, cells proliferating in YEPD without isoamyl alcohol displayed a single large DAPI-staining entity which, in a few cells with very large buds, could be seen stretched through the neck from the mother into the bud (not shown). Clearly, the hyphal-like extensions develop via bud initiation and elongation and undergo DNA replication and nuclear division, but, from many considerations, they are obviously neither normal 'yeast cells' nor 'pseudohyphal cells'.

It is informative to detail some further features of the hyphal-like extensions formed in the presence of 0.5% isoamyl alcohol. Firstly, mutations in any of the genes *STE7*, *STE11*, *STE12* or *STE20* in homozygous diploids had no effect on the ability to form hyphal-like extensions. This could be taken to imply that the formation of hyphal-like extensions and pseudohyphae are unrelated. However, it could also be argued that isoamyl alcohol is interacting with this pathway 'below' *STE12*. A number of the presumed elements of the pseudohyphal development pathway remain to be identified (e.g. the unique mitogen-activated protein kinase and a cyclin-dependent kinase inhibitor). Perhaps isoamyl alcohol is interacting with one of these functions. A further possibility is another separate pathway. If this seems improbable, it should be remembered that a second osmosensing signal-transduction pathway was discovered in *S. cerevisiae* very recently (Davenport *et al.*, 1995). Secondly, isoamyl alcohol does not appear to act primarily by causing unipolar development. There is no way that unipolar development could generate forms such as those in Fig. 1(c). Thirdly, when returned to fresh liquid medium or placed on YEPD agar, the 'yeast cell' ends of structures bearing hyphal-like extensions resumed 'yeast cell' proliferation (via budding and the production of smaller daughters). Nucleated portions of the hyphal-like extensions proliferated via one or two cycles of pseudohyphal pattern growth and then reverted to 'yeast cell' proliferation. Non-nucleated portions (such as the simple extension in Fig. 1b, which is without a bulb separated by a partial constriction, as seen in Fig. 1c), could not proliferate.

On the other hand, incubation of *S. cerevisiae* in YEPD containing only 0.25% (v/v) isoamyl alcohol led to the formation of true pseudohyphae (Fig. 3). (In the case of haploids these should be called 'haploid filament cells'.) The filament cells (pseudohyphal cells in diploids) were regular in shape, longer than 'yeast cells' and had synchronous branching patterns. As one might expect, the chains observed in vigorously shaken liquid cultures were not as long as those formed on solid media; i.e. they comprised fewer cells. Nevertheless, they had all the other properties associated with filament cells: they all reverted to 'yeast cells' when washed and returned to liquid YEPD medium lacking isoamyl alcohol and were all able

Fig. 3. Haploid *S. cerevisiae* strain IWD72 after incubation in YEPD medium containing 0.25% (v/v) isoamyl alcohol. By 3 h, the majority of cells were in clusters or strings, but even where cells remained as pairs, the presence of equal-sized buds suggests the cell cycles had become synchronized (a, b). In (a), the pair of equal-sized mothers with equal-sized buds indicates that the mothers' cycles were also synchronized. By 4 h, each group or cluster of cells contained elongated individuals (indicated by arrows) characteristic of those in invasive haploid filaments on solid media (c). Bar, 40 μ m.

to found fresh colonies on agar. Roberts & Fink (1994) reported that the cells in diploid pseudohyphal filaments are more elongated than those in haploid filaments; the same was true of those formed in the presence of 0.25% (v/v) isoamyl alcohol.

Other 'fusel' alcohols induce hyphal-like extensions in *S. cerevisiae*

S. cerevisiae produces a number of 'fusel' alcohols as the result of its metabolism (Webb & Ingraham, 1963). Thus it seemed worthwhile examining whether a range of other alcohols induced morphological change when included in YEPD medium. All of these compounds were tested at 0.5% (v/v), except for *n*-hexanol, which was added at 0.1% (v/v) because it proved to be toxic above 0.2%, and 2-phenylethanol, which was added at 0.25% because it was also toxic at higher concentrations. Similar levels of hyphal-like extensions were formed in the presence of *n*-amyl alcohol and isoamyl alcohol (Table 4). Active amyl alcohol and isobutanol resulted in moderate amounts of hyphal-like extensions. With *n*-propanol, *n*-butanol and *n*-hexanol, very low levels of extensions were formed. No extensions were formed in the presence of propan-2-ol or 2-phenylethanol (Table 4).

The observation that hyphal-like extensions were formed in ethanol minimal medium only with leucine as nitrogen source and not with isoleucine or valine (Dickinson, 1994) must be because the catabolism of leucine leads to the

Table 4. Effects of various short-chain alcohols on yeast morphology

Prototrophic haploid IWD72 was grown in YEPD to OD₆₀₀ 10.0. Aliquots were removed and inoculated into fresh 100 ml batches of YEPD medium containing different short-chain alcohols. The results (scored after 24 h) are the means of two separate experiments; standard errors were < 4%.

Alcohol	Hyphal-like extensions in culture (%)
None (control)	0.0
<i>n</i> -Propanol	5.0
Propan-2-ol	0.0
<i>n</i> -Butanol	1.0
Isobutanol	15.5
<i>n</i> -Amyl alcohol	75.0
Active amyl alcohol	24.5
<i>n</i> -Hexanol	4.0
2-Phenylethanol	0.0

formation of isoamyl alcohol (Table 1; and Webb & Ingraham, 1963). The observation that *n*-amyl alcohol also induces high levels of hyphal-like extensions is presumably a consequence of structural similarity between isoamyl alcohol and *n*-amyl alcohol. Since *n*-amyl alcohol can be derived from threonine (Webb & Ingraham, 1963), one might expect that culturing yeast in ethanol minimal medium plus threonine would result in the formation of hyphal-like extensions. This did not happen in any strain tested; the cells underwent five doublings and formed *n*-amyl alcohol but did not form hyphal-like extensions. This result should be treated with caution because variation between strains in the ability to serve as sole source of nitrogen seems to be greater in the case of threonine than for any other amino acid (Cooper, 1982). Perhaps all of the strains which I have examined do not utilize threonine efficiently.

The 'fusel' alcohols which induced moderate levels of extensions (active amyl alcohol and isobutanol) are derived from isoleucine and valine, respectively. The fact that the levels of these two alcohols were both much lower in media after yeast had been cultured in the presence of isoleucine or valine than the level of isoamyl alcohol when leucine was the nitrogen source (Table 1), and the fact that they induce less formation of extensions, probably explains why these structures were not observed in ethanol minimal medium containing isoleucine or valine.

Addition of *n*-propanol, *n*-butanol and *n*-hexanol all resulted in very low levels of hyphal-like extensions. The two former compounds are said to be derived from threonine (Webb & Ingraham, 1963); the metabolic origin of *n*-hexanol is obscure. Propan-2-ol and 2-phenylethanol did not affect yeast morphology. Propan-2-ol is not a 'fusel' alcohol, whereas 2-phenylethanol is considered to be one. The fact that propan-2-ol and 2-

Table 5. Effects of isoamyl alcohol on the morphology of some yeasts

The various organisms were grown in YEPD to OD₆₀₀ 10.0. Aliquots were removed and inoculated into fresh 100 ml batches of YEPD medium containing isoamyl alcohol to give an OD₆₀₀ of 0.1. Isoamyl alcohol was used at 0.55% (v/v) with *C. boidinii* and *B. anomalus* and at 0.6% (v/v) with *C. albicans*. The results (scored after 24 h) are the means of two separate experiments; standard errors were ≤ 3%.

Organism	Hyphal-like extensions (%)	Pseudohyphae (%)
<i>C. albicans</i>	0	77
<i>C. boidinii</i>	0	80*
<i>B. anomalus</i>	3	6

* In the absence of isoamyl alcohol, cultures of *C. boidinii* contained 2.5% pseudohyphae at this time, whereas control cultures of the other two yeasts were devoid of both pseudohyphae and hyphal-like extensions.

phenylethanol had no effect on morphology and that neither of them are related to branched-chain amino acid catabolism is perhaps not coincidental.

Isoamyl alcohol induces pseudohyphal formation in several species of yeasts

Isoamyl alcohol induced the formation of pseudohyphae (i.e. chains of uniformly shaped cells) in *C. boidinii*, *C. albicans* and *B. anomalus* (Table 5). It also resulted in the formation of low levels of hyphal-like extensions in *B. anomalus*. The fact that (dependent upon concentration) isoamyl alcohol induced hyphal-like extensions in two yeasts, pseudohyphae in all four species and both in two of them indicates a close similarity between the two processes. It also suggests that yeast cells deploy some cellular structures of proteins for a variety of end uses (e.g. cell cycle functions for differentiation and vice versa). Such a notion is not without precedent, for it now seems increasingly likely that some portions of the signalling pathways (for mating, pseudohyphal development, cell integrity, high osmolarity glycerol response and sporulation) in *S. cerevisiae* cross-communicate (Levin & Errede, 1995).

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