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Pichia fermentans dimorphic changes depend on the nitrogen source

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ABSTRACT

Pichia fermentans DiSAABA 726 is a biofilm-forming yeast that undergoes dimorphic transition. Under yeast-like morphology it controls brown rot caused by *Monilia* spp. on apple fruit, while under pseudohyphal form, it shows pathogenic behaviour itself on peach fruit. The present study investigates the nutritional factors that induce and separate yeast-like and pseudohyphal morphologies under laboratory conditions. We show that *P. fermentans* DiSAABA 726 produces mainly yeast-like cells on media containing millimolar concentrations of urea and diammonium phosphate, and forms pseudohyphae at micromolar concentrations of these two salts. With ammonium sulphate, yeast-like or pseudohyphal morphology depends on the N concentration and the pH of the culture media. Amino acids such as methionine, valine, and phenylalanine invariably induce pseudohyphal morphology irrespective of the N concentration and the pH of the culture media. Methionol, 1-butanol, isobutanol, and isopropanol induce pseudohyphal growth, while phenylethanol and isoamyl alcohol fail to induce the formation of filaments. Thus, the morphogenesis of *P. fermentans* DiSAABA 726 depends more on the nitrogen source than on the N concentration, and is regulated by the quorum-sensing molecules that are generally produced from amino-acid assimilation under nitrogen starvation.

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Introduction

Numerous fungi, including *Saccharomyces cerevisiae*, *Candida albicans*, *Cryptococcus neoformans*, *Ustilago maydis*, *Pichia fermentans*, *Schizosaccharomyces japonicus*, *Yarrowia lypolitica*, and others, switch between yeast-like and pseudohyphal and/or hyphal morphologies in response to nutritional and environmental stimuli (Madhani & Fink 1998; Sipiczki & Takeo 1998; Sanchez-Martinez & Perez-Martin 2001; Herrera & Sentandreu 2002). In the yeast-like form mitotic division by budding or fission produces independent daughter cells. In the pseudohyphal form the cells are generally elongated, remain attached, and produce branched chains that show marked constrictions between the cells; the hyphal form is characterized by the presence of true septa and a lack of constrictions between the cells (Yarrow 1998). Pseudohyphal and hyphal forms provide selective advantages to nonmotile yeast cells by facilitating their escape from hostile environments and by allowing their exploration of the surrounding space for nutrient scavenging. Moreover, pseudohyphae and hyphae can penetrate the surface on which these fungi grow, and their formation is often associated with

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pathogenic behaviour (Saville *et al.* 2003; Whiteway & Oberholzer 2004).

For this reason the nutritional and environmental stimuli that induce hyphal/pseudohyphal growth have been extensively studied in many different fungal models, which have included *S. cerevisiae* and *C. albicans*. Both of these microorganisms can switch from yeast-like to pseudohyphal forms in response to nitrogen starvation (Gimeno *et al.* 1992; Wickes *et al.* 1996; Csank & Haynes 2000; Biswas & Morschhäuser 2005). Dimorphic transition can also be triggered by carbon starvation, changes in pH and temperature, and production of quorum-sensing molecules, which include the higher alcohols (Dickinson 1994; Dickinson 1996; Lorenz *et al.* 2000; Maidan *et al.* 2005; Chen & Fink 2006).

In spite of the many different signals that can induce filamentous development, the signalling pathways involved in the integration of external stimuli and cell differentiation have been well conserved among distantly related fungi (Madhani & Fink 1998; Sudbery *et al.* 2004). In particular, the mitogen-activated protein kinase (MAPK) and the cAMP–protein kinase A (PKA) pathways are involved in the induction of hyphal growth in both *C. albicans* and *S. cerevisiae*, and in *S. cerevisiae*, the *FLO*11 gene is the final target of these two signal-transduction pathways (Lo & Dranginis 1998; Rupp *et al.* 1999). Moreover, the involvement of the ammonium and amino-acid sensing pathways in pseudohyphal growth has been reported for *S. cerevisiae* and *C. albicans* (Lorenz & Heitman 1998; Klasson *et al.* 1999; Pan *et al.* 2000; Biswas & Morschhäuser 2005). In *S. cerevisiae* ammonium permease is encoded by *MEP2*, and it is involved in signal transduction under low ammonium concentrations (Lorenz & Heitman 1998). In *C. albicans*, CaMep2p induces the formation of filaments in response to ammonium and amino-acid starvation (Biswas & Morschhäuser 2005).

The yeast *P. fermentans* DiSAABA 726 controls brown rot caused by *Monilinia* spp. on apple fruit but it shows pathogenic behaviour on peach fruit also in the absence of *Monilinia* spp. (Giobbe *et al.* 2007). In both cases, *P. fermentans* forms a biofilm on the fruit surface: on apple, this is made of yeast-like cells, while on peach, it consists of pseudohyphae. Although the formation of pseudohyphae is often considered a virulence factor, a clear relationship between *P. fermentans* pseudohyphal growth and its pathogenic behaviour on peach fruit has not been demonstrated to date. Here, within the frame of a project aimed at understanding whether dimorphic transition has to be considered a risk factor associated to the utilization of microbial antagonists in postharvest, the objective of the present work was the identification of the nutritional factors that can induce and separate the *P. fermentans* yeast-like and pseudohyphal morphologies under laboratory conditions.

Materials and methods

Strain and culture conditions

Pichia fermentans Lodder [anamorph: *Candida lambica* (Lindner & Genoud) Uden & H.R. Buckley ex S.A. Mey. & Ahearn (1983)] DiSAABA 726 (=DBVPG 3627) was isolated from wine must and maintained in the culture collections of the Dipartimento di Scienze Ambientali Agrarie e Biotecnologie AgroAlimentari in YEPD (2 % glucose, 2 % peptone, 1 % yeast extract, 2 % agar) at 4 °C for short-term storage, and in YEPD plus 20 % glycerol at −80 °C for long-term storage.

The other culture media used were: YNB-based media (0.67 % Yeast Nitrogen Base [Difco], plus 0.5 %, and 2.0 % glucose or fructose, as required); SLAD (0.2 % Yeast Nitrogen Base without amino acids and ammonium sulphate, plus 50 μM ammonium sulphate, and 2 % glucose); and YCB-based media (1.17 % Yeast Carbon Base, plus urea, ammonium phosphate, ammonium sulphate or amino acids, as the sole nitrogen sources, as required).

YEPD and SLAD were also used with the addition of 1 % methionol, isobutanol, isoamyl alcohol, isopropanol or 1-butanol, or 0.25 % phenylethanol. Citrate buffer (0.1 M citric acid, 0.1 M sodium citrate, pH 6) was also added to the YCB-based culture media that contained urea, ammonium sulphate, ammonium phosphate, methionine, valine, and phenylalanine, as required. All of the media were used with 2 % agar, unless otherwise specified. *Pichia fermentans* DiSAABA 726 was precultured overnight at 25 °C in YEPD. The cells were then rinsed twice in sterile distilled water, inoculated on solid (5×10^6 cells) or liquid (5×10^6 cells ml^{-1}) media, and incubated statically at 25 °C.

Isolation of single yeast cells and pseudohyphae

Single yeast cells and pseudohyphae were sampled using a Singer MSM micromanipulator (Singer Instruments Co. Ltd., Somerset, UK), plated onto either YCBU (YCB plus 0.2 % or 0.5 % urea) or YCBM (YCB plus 0.2 % methionine), and incubated at 25 °C while being observed for up to 3 d.

Image analysis

The cells were photographed at 100× magnifications, using an XM10 camera mounted on an Olympus BX61 microscope. Percentages of yeast-like cells and pseudohyphae were evaluated on at least 500 cells sampled from three independent cultures for each growth condition. Images of representative cells are shown.

Results

The nitrogen source dictates the morphology of Pichia fermentans

With the aim of identifying the individual nutritional effectors of dimorphic transition, *P. fermentans* DiSAABA 726 was plated onto a variety of culture media that differed according to the carbon and nitrogen sources (Table 1).

For the majority of these culture media (all of the YNB-based media, and the YCB-based media with added alanine, glycine, isoleucine, histidine, threonine, arginine, asparagine, proline, and trans-proline), *P. fermentans* DiSAABA 726 produced mixtures of yeast-like cells and pseudohyphae (Table 1). Only a few of the media tested led to the segregation of these two morphologies. In particular, on YEPD, YCBU (YCB plus 0.2 % or 0.5 % urea), and YCBphos (YCB plus 0.2 % or 0.5 %

Table 1 – *P. fermentans* DiSAABA 726 cell morphology on the different culture media, according to the medium supplements.

Culture medium	Supplement	Cell morphology
YEPD	–	Yeast-like cells and a few pseudohyphae
SLAD	–	Pseudohyphae
YNB[a]	Glucose: 0.5 %, 2.0 %	Yeast-like cells and pseudohyphae
	Fructose: 0.5 %, 2.0 %	Yeast-like cells and pseudohyphae
YCB[b]	Urea: 0.2 %, 0.5 %	Yeast-like cells
	Diammonium phosphate: 0.2 %, 0.5 %	Yeast-like cells
	Ammonium sulphate: 0.2 %, 0.5 %	Pseudohyphae
	Alanine, arginine, asparagine, glycine; isoleucine, histidine, threonine, serine, lysine: 0.2 %, 0.5 %	Yeast-like cells and pseudohyphae
	Methionine, valine, phenylalanine: 0.2 %, 0.5 %	Pseudohyphae

a YNB contains 0.5 % ammonium sulphate.
b YCB contains 1 % glucose.

diammonium phosphate), *P. fermentans* DiSAABA 726 produced mainly yeast-like cells (Fig 1). On SLAD and on YCB-sulph (YCB plus 0.2 % or 0.5 % ammonium sulphate), YCBM (YCB plus 0.2 % or 0.5 % methionine), YCBV (YCB plus 0.2 % or 0.5 % valine), and YCBP (YCB plus 0.2 % or 0.5 % phenylalanine), *P. fermentans* DiSAABA 726 showed pseudohyphal morphology (Fig 1). Yeast morphology was examined also on liquid YNB and YCB added with all the carbon and nitrogen sources tested. In these culture conditions *P. fermentans* DiSAABA 726 always formed a biofilm at the air liquid interface and showed a cell morphology that was the same observed in solid media showing the same composition (data not shown). Thus, as observed for other yeasts, *P. fermentans* DiSAABA 726 produced mainly yeast-like cells on media

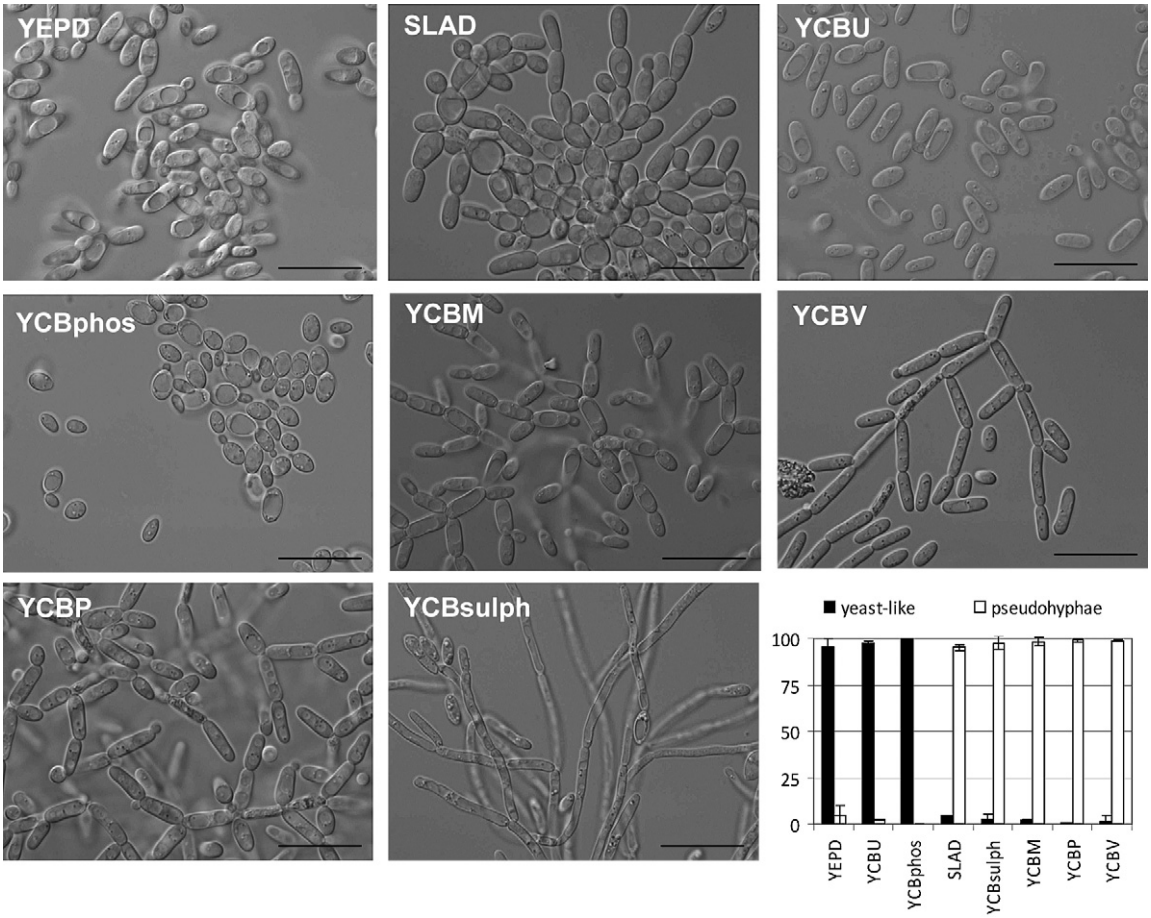

Fig 1 – Cell morphology of *P. fermentans* DiSAABA 726 on culture media containing 0.2 % of the different nitrogen sources. The cells were sampled after 3 d growth on solid media and photographed at 100× magnification. Histogram shows the percentages of yeast-like cells and pseudohyphae on each culture medium. Bars represent standard deviations. Data are representative of at least three independent experiments.

containing readily assimilable nitrogen sources such as yeast extract and peptone, urea or diammonium phosphate. In contrast, pseudohyphal transition was seen with ammonium sulphate (SLAD, YCBsulph) and with specific amino acids (YCBM, YCBV, YCBP) (Gimeno *et al.* 1992; Wickes *et al.* 1996; Csank & Haynes 2000; Biswas & Morschhäuser 2005) although with marked differences on the different nitrogen sources.

To determine whether the concentration of the nitrogen source is important for *P. fermentans* DiSAABA 726 morphogenesis, yeast cells were inoculated on YCB-based media containing increasing concentrations of the nitrogen sources that triggered yeast-like (diammonium phosphate, urea) and pseudohyphal (ammonium sulphate, methionine, valine, phenylanaline) growth. The concentrations chosen were: 50 μM, as the concentration of ammonium sulphate in SLAD; and 13.5 mM and 37 mM, which approximately correspond to the molarities of the amino acids and ammonium salts that were added to the culture media at 0.2 % and 0.5 %, respectively. At the lowest concentration of these nitrogen sources (50 μM), *P. fermentans* DiSAABA 726 produced pseudohyphae (Fig 2). On YCB plus ammonium sulphate, methionine, valine or phenylalanine, pseudohyphal growth persisted also at increasing concentration of the nitrogen source. In contrast, the increased availability of diammonium phosphate and urea inhibited the formation of pseudohyphae, and resulted in yeast-like morphology (Fig 2). Thus, there was a concentration-dependent effect when the nitrogen source was diammonium phosphate or urea, but not when it was ammonium sulphate, methionine, valine or phenylalanine.

However, it needs to be considered that equimolar amounts of the different nitrogen sources that were added to the culture media actually contain different amounts of N as a macronutrient. In particular, the amount of N in YCBphos, YCBsulph, and YCBU 37 mM is double than that contained in YCBM, YCBV, and YCBP 37 mM (Table 2). Thus, this yeast-like morphology on the YCBP and YCBU might arise from the higher concentrations of N. To evaluate this hypothesis, the effects of equal amounts of N on *P. fermentans* DiSAABA 726 morphogenesis were analysed. In particular, considering that '37 mM' YCBphos actually contains 1.03 g L^{-1} N, and that this amount is sufficient to induce yeast-like growth, *P. fermentans* DiSAABA 726 cells were inoculated in culture media containing the same amount of N in the form of urea, diammonium phosphate, methionine, valine, phenylalanine or ammonium sulphate. Here, the yeast morphology indicated that 1.03 g L^{-1} N in form of urea or diammonium phosphate induces the differentiation of yeast-like cells. In contrast, 1.03 g L^{-1} N in form of methionine, valine, phenylalanine or ammonium sulphate induced the formation of pseudohyphae. This confirms that the nitrogen source affects the *P. fermentans* DiSAABA 726 morphology more than the nitrogen concentration (Fig 3).

Extracellular pH interferes with ammonium sulphate assimilation and cell morphology

According to the results illustrated in Figs 2 and 3, ammonium sulphate and diammonium phosphate have different effects on the *Pichia fermentans* DiSAABA 726 morphology. Considering that nitrogen in ammonium salts is easily assimilated by yeast (Bamforth 2005), and that these two salts contain equal amounts of N, this effect might be due to the different buffering capacities of these two salts. To evaluate this hypothesis, *P. fermentans* DiSAABA 726 was inoculated into YCB-based liquid media containing ammonium sulphate and diammonium phosphate at the same concentrations as previously used, without and with the addition of citrate buffer (pH 6.0). Both cell morphology and pH were monitored for up to 3 d.

The results obtained here confirm that the ammonium sulphate buffering capacity is lower than that of ammonium phosphate (Table 3). Moreover in buffered YCBsulph, *P. fermentans* DiSAABA 726 grew with a yeast-like morphology (Fig 4). Therefore, pseudohyphal growth might be a consequence of the strong acidification of the culture media containing millimolar concentrations of ammonium sulphate. The same protocol was thus repeated on nonbuffered and buffered YCBU, YCBM, YCBV, and YCBP. Similar to what was seen for YCBsulph and YCBphos, also on these nonbuffered media there was a progressive decrease in the external pH. However, contrary to what was observed in YCBsulph, the *P. fermentans* DiSAABA 726 morphology was independent of the external pH, as yeast-like on buffered YCBU, and pseudohyphal on buffered YCBM, YCBV, and YCBP (data not shown).

Pichia fermentans *morphogenesis is affected by higher alcohols*

The assimilation of amino acids through the Ehrlich pathway can lead to the production of higher alcohols. As with other yeasts, these higher alcohols might be involved in *P. fermentans* pseudohyphae formation. To evaluate this hypothesis, *P. fermentans* DiSAABA 726 was inoculated onto SLAD and YEPD plus methionol, phenylethanol or isobutanol. Moreover, to further investigate the role of other alcohols that have shown effects on *Saccharomyces cerevisiae* morphogenesis (Lorenz *et al.* 2000), isoamyl alcohol, 1-butanol, and isopropanol were also added to SLAD and YEPD. Across these alcohols, there were different effects seen on *P. fermentans* DiSAABA 726 cell morphology. On SLAD, but not on YEPD, methionol enhanced cell elongation and promoted production of true pseudohyphae. For both media, although phenylethanol induced slight cell elongation and aggregation, it did not result in a clear pseudohyphal morphology; in contrast, isobutanol induced pseudohyphal growth on both media (data not shown). Isoamyl alcohol, which is a known trigger of filament formation in *S. cerevisiae* (Martinez-Anaya *et al.* 2003), did not induce pseudohyphal growth in *P. fermentans* DiSAABA 726. As isoamyl alcohol is a byproduct of leucine assimilation through the Ehrlich pathway, this result is in agreement with the evidence that leucine cannot induce pseudohyphal growth in *P. fermentans* DiSAABA 726. On the contrary, on SLAD and YEPD, 1-butanol and isopropanol both induced pseudohyphal growth (data not shown), and similar to isobutanol, they also acted as triggers of filament formation on nitrogen-rich medium.

Pseudohyphae formation is reversible in Pichia fermentans

The yeast-like cells and pseudohyphae sampled from YCBU and YCBM, respectively, were separated with a micromanipulator and inoculated on both YCBU and YCBM. This resulted in

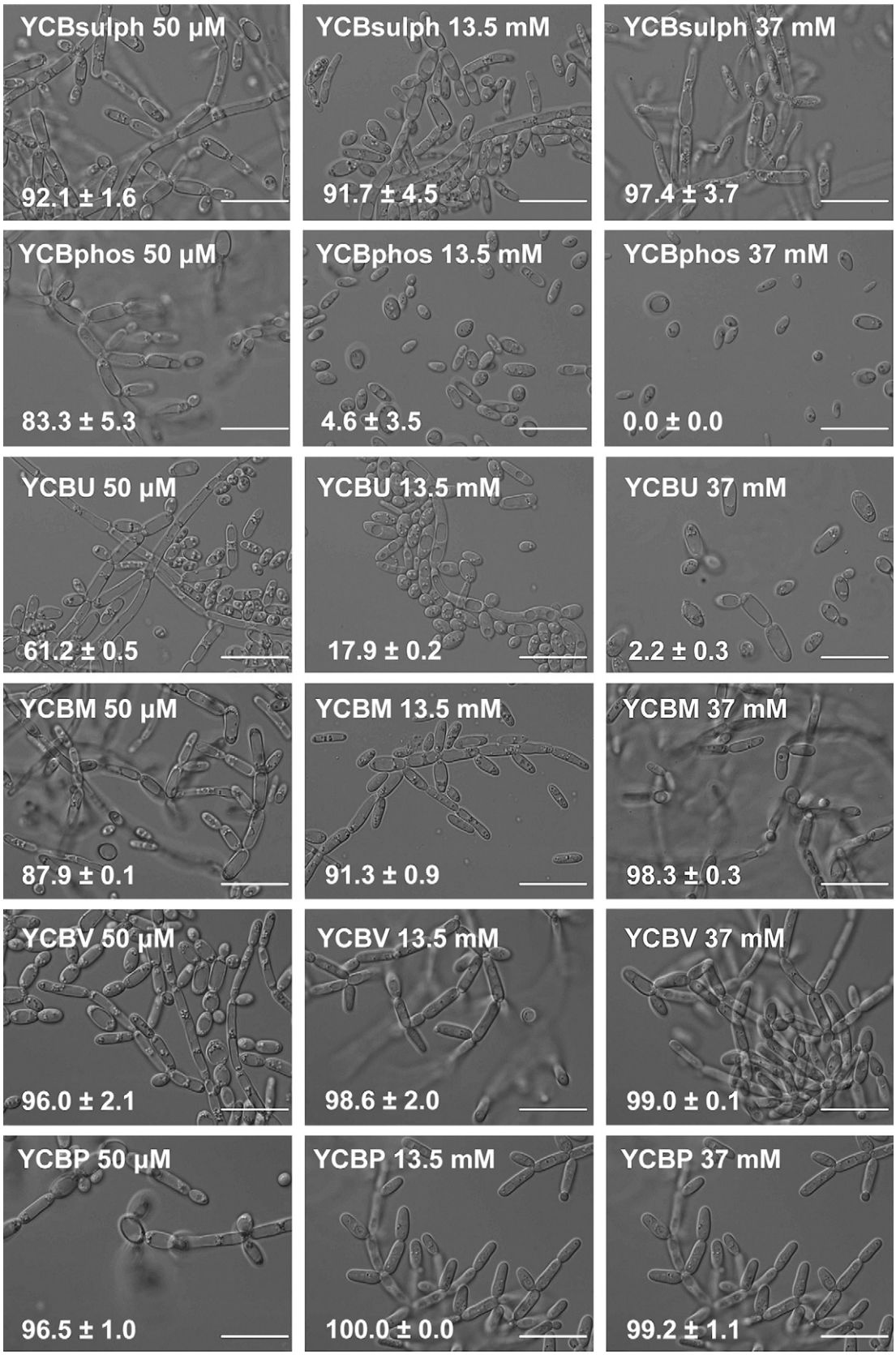

Fig 2 – Representative images of *P. fermentans* DiSAABA 726 cell morphology on YCB plus increasing concentrations of ammonium sulphate, diammonium phosphate, urea, methionine, valine, and phenylalanine, as indicated. The cells were sampled after 3 d growth on solid media and photographed at 100× magnification. Percentages of pseudohyphae on total cells are reported. Bars, 20 μm.

Table 2 – Amount of the macronutrient N in the culture media containing 37 mM of the different nitrogen sources.

Nitrogen source (37 mM)	Nitrogen amount (g L^{-1})
Urea	1.03
Diammonium phosphate	1.03
Ammonium sulphate	1.06
Methionine	0.52
Valine	0.52
Phenylalanine	0.52

the yeast-like cells maintaining their morphology on YCBU but producing pseudohyphae on YCBM. Similarly, the pseudohyphae shifted to yeast-like cells on YCBU, while maintaining their pseudohyphal morphology on YCBM (data not shown). Thus, as has been seen for dimorphic transition with other yeasts, *P. fermentans* DiSAABA 726 shows a completely reversible morphology that strictly depends on the available nitrogen source.

Discussion

Pseudohyphae formation is described, although not extensively studied, in different species of the genus *Pichia* (Kurtzman *et al.* 1980; Kurtzman 2000; Bhadra *et al.* 2007; Oberoi *et al.* 2012). For what concerns *Pichia fermentans*, the coexistence of yeast-like cells and pseudohyphae on the majority of the culture media indicates that growth with a mixed morphology is a natural condition for this dimorphic yeast. Similar to what has been observed for other yeasts, dimorphic switch in *P. fermentans* might also be functional, relating to the need for yeast dissemination or nutrient scavenging under different growth or environmental conditions. However, as pseudohyphal growth in *P. fermentans* DiSAABA 726 appears to be associated with a pathogenic behaviour (Giobbe *et al.* 2007), the definition of the factors that trigger its pseudohyphal transition is of particular interest.

The data obtained in the present study indicate that separation of the yeast-like and pseudohyphal morphologies can

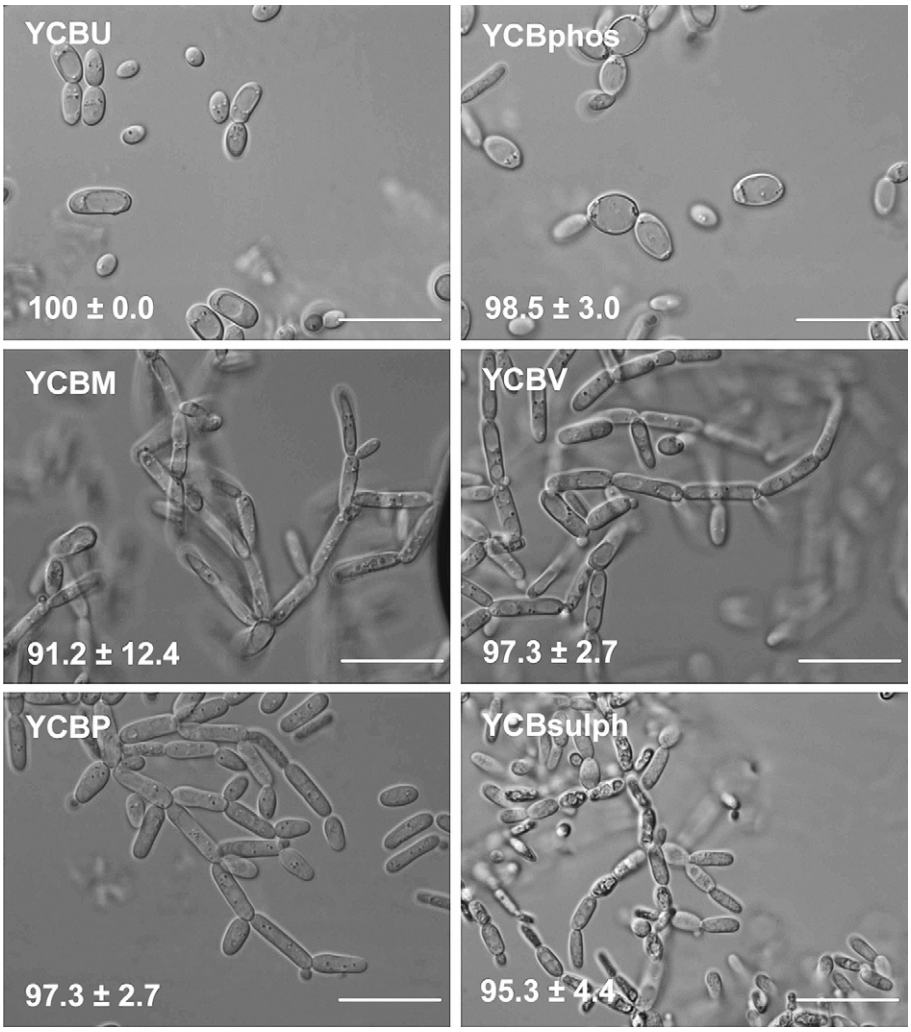

Fig 3 – Representative images of *P. fermentans* DiSAABA 726 cell morphology on YCB plus 1.03 g L^{-1} N in form of urea, diammonium phosphate, methionine, phenylalanine, valine, and ammonium sulphate, as indicated. The cells were sampled after 3 d growth on solid media and photographed at 100× magnification. Percentages of yeast-like cells (YCBU, YCBphos) or pseudohyphae (YCBM, YCBP, YCBV, YCBsulph) on total cells are reported. Bars, 20 μm.

Table 3 – pH of the nonbuffered and buffered liquid culture media at the start (time 0) and end (time 72; 72 h) of the *P. fermentans* DiSAABA 726 cultures.

Culture medium	Conc. added	pH			
		Nonbuffered		Buffered	
		Time 0	Time 72	Time 0	Time 72
YCBU	50 μM	5.79	3.70	6.00	5.93
	13.5 mM	5.76	5.18	6.00	6.00
	37 mM	5.72	5.52	6.00	5.92
YCBM	50 μM	5.76	4.13	6.00	5.87
	13.5 mM	5.74	3.63	6.00	4.98
	37 mM	5.86	3.62	6.00	4.91
YCBphos	50 μM	5.84	4.03	6.00	5.83
	13.5 mM	7.00	5.83	6.00	5.28
	37 mM	7.48	6.75	6.00	4.83
YCBsulph	50 μM	5.74	4.15	6.00	5.94
	13.5 mM	5.68	2.52	6.00	4.93
	37 mM	5.74	4.15	6.00	5.94
YCBV	50 μM	5.50	3.37	6.00	5.30
	13.5 mM	5.52	3.41	6.00	5.57
	37 mM	5.55	3.70	6.00	5.73
YCBP	50 μM	5.75	4.50	6.00	5.90
	13.5 mM	5.60	3.44	6.00	5.14
	37 mM	5.64	3.56	6.00	5.10

be achieved through the use of specific nitrogen sources. The induction of either one or the other of these morphologies is also completely reversible, as has been seen for the opportunistic pathogen *Candida albicans* (Sanchez-Martinez & Perez-Martin 2001). Indeed, among the nutritional factors involved in *P. fermentans* morphogenesis, the nitrogen source has an important role.

However, while the induction of the yeast-like morphology by ammonium phosphate and complex nitrogen sources (yeast extract and peptone) is compatible with their straightforward assimilation, the effects of urea, ammonium sulphate, methionine, valine, and phenylalanine as the nitrogen source require further explanation. The formation of pseudohyphae at 50 μM of each of these nitrogen sources used is consistent with the known effects of nitrogen starvation on yeast morphogenesis. More intriguing is the yeast-like morphology of *P. fermentans* DiSAABA 726 on millimolar concentration of urea.

Urea is generally considered a poor nitrogen source. In *C. albicans* and other pathogenic yeasts, urea induces the formation of filaments (Ghosh *et al.* 2009), and the yeast-like to pseudohyphae transition is mediated by the CO_2 produced during urea assimilation. After being converted into bicarbonate by carbonic anhydrase, CO_2 activates, in sequence: adenylyl cyclase, cAMP-dependent PKA, and hypha-specific genes (Bahn & Mühlschlegel 2006; Elleuche & Pöggeler 2010; Hall *et al.* 2010). On this basis, the lack of filament formation during growth on YCB with millimolar concentrations of urea was rather unexpected, and suggests that the mechanism described in *C. albicans* does not work in the same way in *P. fermentans* DiSAABA 726. This might be because during growth on YCBU, CO_2 is produced below a critical threshold for filament formation, and/or that upon conversion into HCO_3^-, the CO_2 is used to fulfil the metabolic demand (Aguilera *et al.* 2005; Elleuche & Pöggeler 2010).

Also, the different effects on the yeast morphology of the millimolar concentrations of diammonium phosphate and ammonium sulphate are particularly interesting. A similar result was seen in *Aureobasidium pullulans*, which undergoes

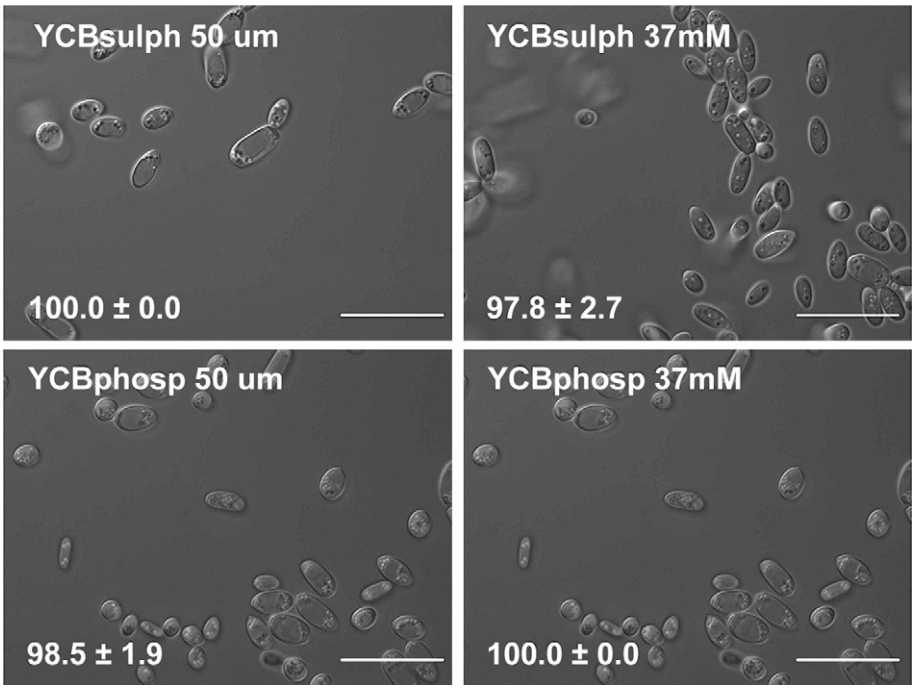

Fig 4 – Representative images of *P. fermentans* DiSAABA 726 cell morphology on buffered YCB plus ammonium sulphate and diammonium phosphate. The cells were sampled after 3 d growth on solid media and photographed at 100× magnification. Percentages of yeast-like cells on total cells are reported. Bars, 20 μm.

transition from yeast-like cells to chlamydospores in ammonium sulphate, and which maintains the yeast-like form in ammonium phosphate (Bermejo *et al.* 1981); this is due to the different buffering capacities of these two salts. Indeed, during growth on liquid YCBsulph, *P. fermentans* DiSAABA 726 showed a dramatic decrease in extracellular pH. Considering that at low pH ammonium uptake occurs *via* an ATP-consuming H^+ antiport transport system (de Morais 2003), this cellular response of *P. fermentans* DiSAABA 726 to ammonium sulphate might be a consequence of a decrease in the uptake of ammonium.

For the effects triggered by methionine, valine, and phenylalanine, it is well known that under a restricted nitrogen supply, their assimilation can occur through the Ehrlich pathway, thus leading to the production of higher alcohols (Jones & Pierce 1964; Henry *et al.* 2007; Hazelwood *et al.* 2008). These higher alcohols may themselves regulate yeast morphogenesis, although with significant differences across the yeasts (Lorenz *et al.* 2000; Martins *et al.* 2007). For example, the isobutanol, phenylethanol, and tryptophol that can derive from valine, phenylalanine, and tryptophan, respectively, can trigger a morphogenetic response in *Saccharomyces cerevisiae* (Lorenz *et al.* 2000; Chen & Fink 2006), with the tyrosol that derives from tyramine having the same effect in *C. albicans* (Chen *et al.* 2004). The effects of methionol and isobutanol are compatible with the hypothesis that methionine and valine can trigger pseudohyphal growth following their transformation into these corresponding higher alcohols. However, isobutanol was effective in both nitrogen-poor and nitrogen-rich media, which might well be due to its 'misleading' effects on the *P. fermentans* nitrogen-sensing pathway (Lorenz *et al.* 2000). In contrast, as seen in *S. cerevisiae* for other higher alcohols (Chen & Fink 2006), methionol appears to be a quorum-sensing molecule only during nitrogen restriction (SLAD), and not in nitrogen-rich medium. According to Lorenz *et al.* (2000), an explanation for this behaviour is that the more rapid growth in the rich medium covers any pseudohyphae formation.

As with reports for *C. albicans* and *Candida dubliniensis* (Martins *et al.* 2007), phenylethanol failed to induce pseudohyphal growth in *P. fermentans* DiSAABA 726. Indeed, *P. fermentans* is a natural producer of this higher alcohol (Jolly *et al.* 2006; Domizio *et al.* 2011), although this phenylethanol production has never been related to pseudohyphal growth in this yeast. Thus, the effects of phenylalanine on *P. fermentans* DiSAABA 726 morphology might not occur through production of phenylethanol.

Pichia fermentans DiSAABA 726 also shows similarities to *C. albicans* and *C. dubliniensis* in terms of the cell response to isoamyl alcohol. In *S. cerevisiae*, isoamyl alcohol induces filament formation through phosphorylation of Cdc28 by Swe1p (Martinez-Anaya *et al.* 2003). This last protein, Swe1p, is not involved in filament formation in *C. albicans* (Whightman *et al.* 2004), which, like *P. fermentans*, maintains a yeast-like morphology also in the presence of isoamyl alcohol.

Similar to the report by Lorenz *et al.* (2000) in haploid cells of *S. cerevisiae*, 1-butanol and isopropanol induced filamentous growth in *P. fermentans* DiSAABA 726, both in nitrogen-poor and nitrogen-rich media. This is in agreement with the morphogenetic effects exerted by different higher alcohols on dimorphic yeasts.

In summary, possibly due to the need to rapidly adapt to nutritional and environmental conditions, *P. fermentans* DiSAABA 726 shows a reversibly changing morphology that depends on the nitrogen source. As equimolar amounts of nitrogen promote yeast-like or pseudohyphal growth according to the nitrogen source, this is compatible with the hypothesis that the nitrogen source is more important than the nitrogen concentration for *P. fermentans* DiSAABA 726 morphogenesis. The effects exerted by the higher alcohols on this yeast indicate that morphogenesis is also regulated, in a species-specific fashion, by the quorum-sensing molecules that are generally produced from amino acids under nitrogen starvation. The results here presented define the cultural conditions that induce and separate yeast-like and pseudohyphal morphologies in *P. fermentans* DiSAABA 726 under laboratory conditions. Indeed at the present stage of the work these results cannot be related to N composition or pH of peach fruit. However they are the starting point for further studies aimed at elucidating the molecular mechanisms involved in dimorphic transition and the risk factors associated with the utilization of dimorphic microbial antagonists in postharvest.

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