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SHORT COMMUNICATION

Glycine oxidation and conversion into amino acids in *Saccharomyces cerevisiae* and *Candida albicans*

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Abstract Humans are exposed much more often to exogenous *Saccharomyces cerevisiae* (a baker's yeast) than exogenous *Candida albicans* (a highly infectious yeast) but suffer no apparent complications from *S. cerevisiae*. We hypothesize that variations in characteristics between these two species may be due, in part, to differences in glycine metabolism. In this study, we examined differences in glycine oxidation between *C. albicans* and *S. cerevisiae*. Both *C. albicans* and *S. cerevisiae* were cultured in glycine enriched media, followed by determination of glycine oxidation and amino acid concentrations in cells. Glycine was degraded to a much greater extent in *C. albicans* than in *S. cerevisiae*. Threonine concentrations and glycine oxidation were also elevated in *C. albicans*. Almost all of the disappearance of glycine from incubation media was accounted for by the formation of serine, threonine, and CO_2 in *S. cerevisiae*, whereas these products represented only 50% of the metabolized glycine in *C. albicans*. The unidentified metabolites of glycine in *C. albicans*, presumably purines, could contribute to its infectious capacity and this warrants further study.

Keywords *Candida albicans* · *Saccharomyces cerevisiae* · Glycine · Serine · Threonine · Metabolism

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Abbreviations

CA	*C. albicans*
SC	*S. cerevisiae*
−O	Anaerobic
+O	Aerobic

Introduction

Saccharomyces cerevisiae (*S. cerevisiae*), sometimes referred to as baker's yeast, is a historically important, benign species of yeast in food industry. In contrast, *Candida albicans* (*C. albicans*) is the primary cause of most medical yeast infections comprising up to 80% of diagnosed infections (Willis 1996). Due to the large number of grain- and cereal-fermented products that humans typically consume, humans are exposed much more often to exogenous *S. cerevisiae* than exogenous *C. albicans* but suffer no apparent infections or complications from this exposure.

Glycine is a small, achiral amino acid that plays an important role in one carbon metabolism of cells (Piper et al. 2000) and thus plays an important role in supporting cell division (Wu 2009). The three primary routes of glycine metabolism are serine formation via serine hydroxymethyltransferase, oxidative decarboxylation via the glycine cleavage system (glycine synthase), and nucleotide base formation (Sinclair and Dawes 1995). Serine serves as an important glycolytic fuel and the products of glycine oxidative decarboxylation provide reductive power (NADH) and one carbon precursors (methylene tetrahydrofolate) for anabolic synthesis.

Based on these observations, we hypothesize that variations in characteristics between these two species may be

due, in part, to differences in glycine metabolism. To test this hypothesis, yeasts were cultured in glycine enriched media, followed by measurements of amino acid production and consumption, as well as CO_2 production from glycine.

Materials and methods

Reagents and solutions

Chemicals were obtained from Sigma Chemical Company (St. Louis, MO) except where noted. Yeast culture and incubation media consisted of yeast nitrogen base supplemented with 1 mM glycine and 20 g/l glucose.

Yeast strains

The yeast strains used in this study were a wild type strain of *S. cerevisiae* ATCC 2601 and a hospital urinary tract isolate of *C. albicans*. The *C. albicans* species was first identified using a serum germ tube test and subsequently confirmed using antiserum specific for *C. albicans*.

Culture conditions and cell harvesting

Yeast cultures (50 ml) were grown aerobically to mid-log phase at 30°C for approximately 16 h as described by Sinclair and Dawes (1995). This method of yeast culture and incubation conditions was chosen because it has previously been used in several studies to analyze glycine metabolism in yeast (Ogur et al. 1977; Sinclair and Dawes 1995; Piper et al. 2000). Cells were harvested by centrifugation at 2,600*g* for 10 min. Cells were resuspended in yeast culture media, centrifuged and resuspended in culture medium. Resuspended cells were counted using a hemacytometer and were appropriately diluted to yield cell samples with approximately the same concentration of cells. Cell numbers were then counted again and used to normalize values of amino acid metabolites.

Incubation conditions

Eight cell samples from each strain were analyzed for metabolism of [U–^{14}C] glycine to L-serine, L-threonine and $^{14}CO_2$. Resuspended yeast cells were incubated in 25 ml center well flasks containing 2 ml culture media supplemented with [U–^{14}C]glycine as described by Ogur et al. (1977) except that NSC tissue solubilizer (Amersham Inc., Arlington Heights, IL) was used to collect $^{14}CO_2$ (Flynn and Wu 1997). Aerobic incubations were flushed with 95/5% oxygen/carbon dioxide and anaerobic incubations were flushed with nitrogen for 10 s prior to being stoppered. Incubations were allowed to occur for 1 h at 37°C and were then terminated by the addition of 200 µl of 1.5 M $HClO_4$. 100 µl of NCS® tissue solubilizer was added to the center well and $^{14}CO_2$ was collected for 1 h and quantified as previously described (Chen et al. 2009). At the end of this incubation, 100 µl of 2 M K_2CO_3 was added to neutralize incubations for analysis. Rates of glycine oxidation were calculated on the basis of $^{14}CO_2$ production and specific radioactivity of [U–^{14}C]glycine in the incubation medium.

Viability

Yeast viability at the beginning and end of culture incubations was analyzed using trypan blue exclusion and A_{280} following centrifugation at 12,500*g* for 5 min (Radel et al. 2000). Viability was >95% as assessed microscopically using trypan blue exclusion. There were no significant differences in A_{280} between species or culture conditions. Morphological analysis of the *C. albicans* strain demonstrated no germ tube formation under these conditions. Additionally, culture conditions did not appear to alter morphology of the *S. cerevisiae* strain as compared to cultures under standard media conditions.

Amino acid measurements

Amino acid values are based upon the number of cells as described above and subtracted from those values obtained for the zero minute incubation (blank). Amino acid concentrations in incubation media were determined using an established reversed-phase HPLC protocol involving pre-column derivatization with *o*-phthaldialdehyde (Li et al. 2009).

Statistical analysis

Data were analyzed by one way analysis of variance and differences among treatment means were analyzed post hoc using Tukey's HSD. All probability values <0.05 were taken to indicate statistical significance. All statistical analyses were performed using SAS (SAS Institute, Cary, NC).

Results

Substantial amounts of glycine were utilized by cultured yeasts, as indicated by the disappearance of glycine from incubation media (Fig. 1). More glycine disappeared in *C. albicans* cultures than in *S. cerevisiae* cultures under both anaerobic and aerobic conditions. Table 1 summarizes the data on resulting concentrations of serine and threonine following incubation with glycine. Under anaerobic

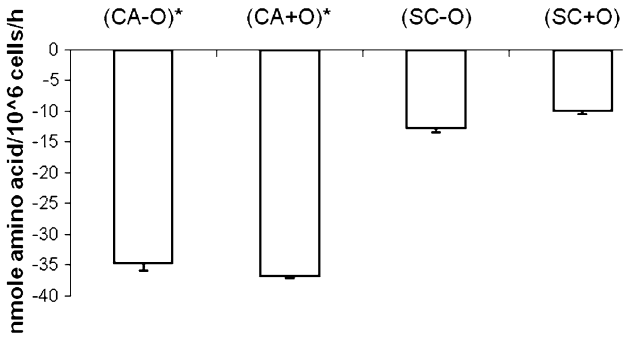

Fig. 1 Glycine disappearance from incubation media. Data were calculated on the basis of glycine concentration in blank incubations subtracted from amino acid concentration in media after incubation. Values are expressed as nmole amino acid/10^6 cells/h. *Asterisk* there was a significant difference ($P < 0.05$) in *C. albicans* or *S. cerevisiae* incubations. *CA C. albicans*; *SC, S. cerevisiae*; *−O*, anaerobic; *+O* aerobic

Table 1 Production of serine and threonine by *C. albicans* and *S. cerevisiae* in glycine enriched media under anaerobic and aerobic conditions

Cell type and conditions	Serine concentration	Threonine concentration
CA, anaerobic	5.08 ± 1.6^{c}	3.99 ± 0.20^{a}
CA, aerobic	8.1 ± 1.1^{b}	3.46 ± 0.30^{a}
SC, anaerobic	12.5 ± 0.7^{a}	0.39 ± 0.09^{b}
SC, aerobic	8.1 ± 0.7^{b}	0.64 ± 0.12^{b}

C. albicans and *S. cerevisiae* were incubated in glycine-rich media under either anaerobic or aerobic conditions. Data for serine and threonine concentrations in cell samples were calculated separately. Data were calculated on the basis of amino acid concentration in blank media subtracted from amino acid concentration in media after incubation. Values are expressed as nmole amino acid/10^6 cells/h

CA, C. albicans; *SC, S. cerevisiae*

Means sharing different superscript letters within each separate column are statistically different ($P < 0.05$)

conditions, *S. cerevisiae* exhibited an increase in serine concentrations when compared to *C. albicans*. Aerobically, there was no difference in the concentration of serine between strains. Regardless of culture conditions, threonine concentrations were elevated in *C. albicans* cultures as compared to *S. cerevisiae*.

There was a difference in glycine oxidation, as measured by $^{14}CO_2$ production, between *C. albicans* and *S. cerevisiae* under both aerobic and anaerobic conditions (Table 2). *C. albicans* consistently oxidized more glycine than *S. cerevisiae* regardless of incubation conditions. For both species, glycine oxidation was lower in the presence of oxygen than in the absence of oxygen.

Discussion

Results of this study indicate, for the first time, a greater rate of glycine utilization in *C. albicans* than in *S. cerevisiae* under these experimental conditions (Fig. 1). Glycine is an important amino acid in one carbon unit metabolism and may be incorporated into nucleotide bases, therefore affecting cell proliferation (Piper et al. 2000). Based on this metabolic role of glycine, we suggest that the overall difference in glycine metabolism may partially explain differences in characteristics between *C. albicans* than in *S. cerevisiae* (Cotter and Kavanagh 2000). The products of glycine metabolism (Fig. 2) provide greater insight into the predominant metabolic pathways in each of the respective species. Examining tetrahydrofolate production by these species is not necessary because, on a stoichiometric basis, tetrahydrofolate was indirectly determined by measuring glycine oxidation ($^{14}CO_2$) and serine production.

The elevated serine concentration in the anaerobic *S. cerevisiae* incubation indicates an increase in serine production from glycine in this species. It is possible that glycine conversion to serine in *S. cerevisiae* outpaces the conversion of serine to pyruvate or other serine-based metabolites. The finding that there was a higher concentration of threonine in *C. albicans* under the same incubation conditions supports this conclusion because serine can serve as a precursor for threonine synthesis in yeast. On the basis of glycine oxidation, it is evident that *C. albicans* is indeed capable of metabolizing more glycine via oxidative pathways than *S. cerevisiae*. Interestingly, this effect was observed under both aerobic and anaerobic conditions.

Another novel and important observation from the present work is that *C. albicans* also exhibited an increase

Table 2 Glycine oxidation by *C. albicans* and *S. cerevisiae* in glycine enriched media under anaerobic and aerobic conditions

	CA − O	CA + O	SC − O	SC + O
nmole Gly/10^6 cells/h	3.21 ± 0.14^{a}	2.63 ± 0.12^{b}	1.99 ± 0.08^{c}	0.93 ± 0.03^{d}

C. albicans and *S. cerevisiae* were incubated for 1 h in glycine-rich media containing [U–^{14}C]glycine under either anaerobic and aerobic conditions. Data were calculated on the basis of specific radioactivity of [U–^{14}C]glycine in the incubation medium

CA, C. albicans; *SC, S. cerevisiae*; *−O*, anaerobic; *+O*, aerobic

Means sharing different superscript letters are statistically different ($P < 0.05$)

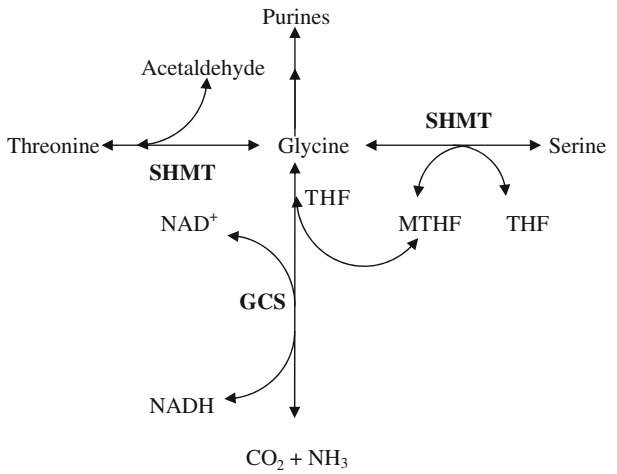

Fig. 2 Pathways of glycine metabolism in yeast. *THF* Tetrahydrofolate, *MTHF* N^5,N^{10}-methylene tetrahydrofolate, *SHMT* serine hydroxymethyltransferase, *GCS* glycine cleavage system

in glycine utilization under aerobic conditions (Fig. 1). Rates of threonine production and glycine oxidation were also higher in *C. albicans* under these conditions. Serine production, however, did not differ between the two species of yeast under aerobic conditions. The decrease in serine production by *S. cerevisiae* under aerobic conditions could be because more serine is converted to pyruvate in this strain compared with *C. albicans*. In contrast, the increase in serine production by *C. albicans* could be explained by the observed decrease in glycine oxidation under aerobic conditions.

Taken together, these findings suggest that *C. albicans* exhibits an increase in glycine metabolism as compared to *S. cerevisiae*. In addition, our results indicate that the metabolism of glycine by *S. cerevisiae* occurs predominately through serine, threonine and oxidative pathways. Almost all of the disappearance of glycine from incubation media in *S. cerevisiae* can be accounted for by the formation of serine and threonine, as well as glycine oxidation on a stoichiometric basis. In contrast, only 50% of the metabolized glycine in *C. albicans* can be accounted for by serine, threonine and oxidative pathways, suggesting that other pathways of glycine metabolism are functioning at a higher rate in this pathogenic species of yeast. The most likely pathway of glycine metabolism that was not examined in this study was purine base synthesis from glycine (Denis 1998). The significance of enhanced purine base production from glycine by *C. albicans* to its pathogenicity certainly warrants further examination.

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