Role of glutathione metabolism status in the definition of some cellular parameters and oxidative stress tolerance of *Saccharomyces cerevisiae* cells growing as biofilms

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Abstract

The resistance of *Saccharomyces cerevisiae* to oxidative stress (H₂O₂ and Cd²⁺) was compared in biofilms and planktonic cells, with the help of yeast mutants deleted of genes related to glutathione metabolism and oxidative stress. Biofilm-forming cells were found predominantly in the G1 stage of the cell cycle. This might explain their higher tolerance to oxidative stress and the young replicative age of these cells in an old culture. The reduced glutathione status of *S. cerevisiae* was affected by the growth phase and apparently plays an important role in oxidative stress tolerance in cells growing as a biofilm.

Introduction

Fungal biofilms have been studied extensively in the last decade (Jirku *et al*., 2001), mainly because they can induce persistent infection and alter biotechnological processes in the food industry. Infections due to disseminating fungal biofilms, especially those involving *Candida albicans*, result in a high mortality rate (>40%) (Wisplinghoff *et al*., 2004). Transition between the planktonic and biofilm states results in numerous alterations of transcriptional activity in *C. albicans* (Kadosh & Johnson, 2005; Murillo *et al*., 2005). The physiology of microorganisms living in the biofilm state differs markedly from that of their planktonic-state counterparts.

Recent reports on the aggregation of *Kluyveromyces lactis* (Coulon *et al*., 2007) and on biofilm formation in *C. albicans* have highlighted the role of glutathione (Murillo *et al*., 2005) and proteins with a high Cys-residue content (Pérez *et al*., 2006). Reduced glutathione (GSH) is the most abundant low-molecular-mass thiol in most living cells (Meister & Anderson, 1983). Because of its low redox potential (E° = −240 mV at pH 7) and high cellular concentration (up to 20 mM), the glutathione disulfide/glutathione (GSSG/2GSH) redox couple largely controls the cellular redox state (Schafer & Buettner, 2001).

*Saccharomyces cerevisiae*, the well-known baker's yeast, can initiate biofilm formation. It adheres efficiently to plastic surfaces, especially when grown in low-glucose media. On semi-solid gels, it forms mats or colonies of a biofilm nature, covering large areas (Reynolds & Fink, 2001). The *S. cerevisiae* genome has been entirely sequenced (Goffeau *et al*., 1996) and various deletion mutants are now available for studying biofilm development genetically.

In the present work, we have used a reference *S. cerevisiae* strain and a series of mutant strains lacking genes involved in glutathione metabolism (synthesis, reduction, transport, degradation) and the oxidative stress response to compare stress tolerance in *S. cerevisiae* grown either as a biofilm or as a planktonic suspension (exponential and stationary...
phases). The results reflect the GSH status and mutation type of the different strains and highlight an important role for glutathione in oxidative stress tolerance in *S. cerevisiae* cells growing as a biofilm.

### Material and methods

#### Strains, growth media, and culture conditions

All strains were isogenic mutants derived from the haploid *S. cerevisiae* strain BY4774 originating from the EURO-SCARF yeast collection (Table 1). The studied mutants lack genes involved in two-step glutathione synthesis (Δgsh1 and Δgsh2), glutathione reduction (Δagr1), vacuolar transport of glutathione and glutathione conjugates (Δycf1), plasma membrane glutathione transport (Δopt1/hgt1), glutathione degradation via γ-glutamyltranspeptidase (GGT) (Δcis2), and the major stress regulon involved in the oxidative stress response (Δyap1).

YPD medium (2% w/v glucose, 2% w/v Bacto peptone, and 1% w/v yeast extract) was used in all experiments under aerobic conditions at 30 °C. For planktonic growth analysis, 1–3 × 10^7 cells from an overnight preculture (about 15 h) were introduced into 100-mL flasks containing 20 mL YPD and continually stirred (100 r.p.m.). Growth was monitored by measuring OD_{600} (data not shown).

*Saccharomyces cerevisiae* biofilm mats were obtained as described previously (Reynolds & Fink, 2001). Phosphate-buffered saline (PBS) solution (10 μL) (it contained 120 mM NaCl, 2.7 mM KCl, and 10 mM K₂HPO₄, and it was adjusted to pH 7.0 with 1 M HCl before sterilization) comprising 1.8–2 × 10^6 cells were spotted at the center of YPD 0.3% agar Petri dishes containing c. 20 mL medium. The growth of cells in mats was monitored by daily counting with a Malassez cell-counting device after suspension in cold PBS. Calculated apparent doubling times were obtained by suspending all yeast cells of a mat in PBS (sacrificial method) before counting. All measurements were performed in triplicate. Doubling times *tₐ* were obtained by plotting log_{10} of the OD (planktonic cultures) or of the number of cells (biofilm-forming cells) vs. the growth time and by applying the formula: *tₐ* = log_{10}2 slope^{-1}.

#### Cell volume determination

Planktonic cells at different stages of growth (exponential and stationary) and the cells of 13-day-old mats were washed twice in cold PBS pH 7.4. Apparent diameters were measured with an optical microscope equipped with a × 100 oil-immersed objective (Olympus BX51). A CCD camera and analysis software were used for image acquisition. Cells were viewed as spheroids with a volume of \( V = \frac{1}{6} \pi a b^2 \), where a and b are, respectively, the major and minor axes of the cells (Wheals, 1982). To avoid optical biases (Gommeaux et al., 2005), measurements were performed after stabilization and sedimentation of the yeast cells; focusing was carried out visually so as to maximize the apparent diameter. The volumes of budded yeasts were calculated by summing the volumes of the buds and the parent cells.

#### Intracellular glutathione concentration measurement

GSH measurements were performed as described previously (Leroy et al., 1993; Saby et al., 1999). Briefly, cells were washed twice in 1 mL cold PBS pH 7.4, resuspended in 1 mL cold 3.3% perchloric acid, and then broken by vigorous vortexing for 3 min. After centrifugation (10 000 g for 15 min at 4 °C), each supernatant containing GSH was distributed into Eppendorf tubes and stored at −80 °C until analysis. GSH was analyzed on an HPLC system coupled with a postcolumn reaction device and spectrophotometric detection. Postcolumn derivatization was performed with ortho-phthalaldehyde. This affords high specificity with respect to other endogenous thiols, except γ-glutamylcysteine (γ-glucys), which reacts in a manner similar to GSH. Easily interpretable chromatograms were obtained, with two baseline-resolved peaks (corresponding to GSH and γ-glucys) and without any late-eluting peaks. Intracellular concentrations were calculated by dividing the GSH concentration of the extracts by the product of the cell number (Malassez counting) and the mean cell volume.
Determination of cell-cycle distribution by flow cytometry

Cells at the different studied stages of growth were centrifuged in cold sterilized ultrapure Milli Q water and stained with 1 μL Syber® Green (Invitrogen) stock solution per 2-mL cell suspension for 15 min at 20 ± 1°C. Fluorescence data were collected on about 10⁵ cells. The fluorescence intensity was plotted on the x-axis and the cell number was plotted on the y-axis. Proportions of G1, S, and G2/M cells were calculated according to Slater et al. (1977).

Disk inhibition assays

Cells were grown at 30 °C in YPD medium from an overnight preculture until they reached the mid exponential phase (OD₆₀₀nm within 0.2) or the early stationary phase (about 12-h-old cultures). Mats were obtained after 13 days at 30 °C as described previously (Reynolds & Fink, 2001). For the inhibition test, 500 μL YPD containing c. 1–2 × 10⁷ cells was mixed with 5 mL soft agar and immediately poured onto YPD-medium-containing Petri dishes. To test tolerance to stress, filter disks were impregnated with 5 μL of 35% (w/w) H₂O₂ or 10 mM CdCl₂ and placed on top of the microbial lawns. Control experiments were conducted with filters soaked in water. Plates were incubated for 48 h at 30 °C before inhibition halo diameters were measured.

Results and discussion

Effect of gene deletion and cell developmental state on growth parameters and cell cycle

In planktonic suspensions and biofilms of S. cerevisiae, we studied the impact of genes involved in glutathione metabolism and the oxidative stress response (Table 1) on growth kinetics, cell volume and number, budding capacity, and cell-cycle distribution. Biofilms formed on semi-solid gels were roughly circular, with a central depression and peripheral lobes (Fig. 1). We did not, however, observe the mature structure described previously (Reynolds & Fink, 2001), where a network of cables forms a central hub with radial spokes emanating from it. Mats covered areas of 5.7–8.8 cm² (mean 7.3 ± 1.7 cm²) after 13 days of growth. In the case of the reference strain and of the cis2, apt1/hgt1, ycf1, and yap1 mutants, the area covered by the mats increased linearly over this 13-day period. In contrast, extension of the area covered by mats of gsh1, gsh2, and glr1 deletion mutants stopped after 11 days. This probably reflects an inadequately maintained redox balance. In all cases, surface cell densities increased dramatically during the first day of growth, from 2.2 ± 1.3 × 10⁶ to 2.2 ± 0.4 × 10⁸ cells cm⁻². Further increase was slower, attaining about 3.4 ± 0.6 × 10⁸ cells cm⁻² by day 8.

Cell volume determinations provided information on cell size distribution in relation to the growth stage and strain deletion (Fig. 2 and Table 2). In planktonic cultures, the average volume was 36.9 ± 13.0 μm³ (n_exp = 1431...
measurements) in the exponential phase ($V_{\text{exp}}$) and 46.3 ± 19.6 µm$^3$ ($n_{\text{stat}} = 1210$ measurements) in the stationary phase ($V_{\text{stat}}$). The corresponding percentages of budded yeast cells were 61.9 ± 3.3% and 28.6 ± 2.8%, respectively. In these planktonic cultures, the tested deletions had no significant influence on either the cell volume or the proportion of budded cells (Table 2).

Likewise, the deletion of genes involved in glutathione synthesis ($gsh1$ and $gsh2$), reduction ($gtr1$), vacuolar transport ($ycf1$), plasma-membrane transport ($opt1/hgt1$), or degradation ($cis2$) or in the oxidative stress response ($yap1$) had very little impact on the growth kinetics of planktonic cultures. All tested strains growing exponentially in rich YPD medium at 30 °C displayed similar doubling times (c. 1.5 h).

In mats, the average cell volume ($V_{\text{biofilm}}$) was 56.9 ± 28.2 µm$^3$ ($n_{\text{stat}} = 1311$ measurements) and the average proportion of budded cells was 14.5 ± 5.3%. Considerable variation in these values was observed according to the strain tested. BY4774 (the reference strain) and.opt1/hgt1 displayed mean cell volumes of 45.8 and 43 µm$^3$, respectively. The $gsh1$, $gsh2$, $gtr1$, and $ycf1$ deletion mutants displayed higher mean cell volumes (59.5, 65.6, 61.7, and 62 µm$^3$, respectively).

The fraction of budded cells was also studied (Table 2). It was approximately twice as high for all strains in the exponential phase as in the stationary phase of planktonic cultures, and it was the lowest in mats, where it varied from 6.9 in the $gsh1$ strain to 18.6 in BY4774 and opt1/hgt1 and up to 22.4% in the $yap1$ strain. It correlated negatively with the mean volume (Fig. 3).

A smaller fraction of budded cells tends to suggest a higher proportion of cells in the G1 phase and a lower proportion in the S phase of the cell cycle. When the cell-cycle distribution of the different cell populations was examined, this tendency was observed to some extent, but not when considering specific deletions (Table 3). Overall, the proportion of cells in G1 was found to increase (and the proportion of cells in S or G2/M to decrease accordingly) from approximately one-third in exponentially growing cells to about two-thirds in mat-forming cells. Differences between strains in terms of cell-cycle distribution were most pronounced in biofilms, where of the five strains ($gsh1$, $gsh2$, $gtr1$, $ycf1$, and $cis2$) showing lower percentages of budding cells than the reference strain and opt1/hgt1, only one ($gtr1$) showed a lower proportion of S-phase cells and none showed a higher proportion of G1-phase cells.

In conclusion, it is clear that constraints on the cell cycle are different for biofilm-forming cells and their planktonic counterparts.

### Effects of gene deletion and physiological state on the level of intracellular reduced glutathione and γ-glucys

Intracellular GSH and γ-glucys concentrations were measured in the S. cerevisiae reference strain BY4774 and its derivatives (Fig. 4). For each strain, in contrast to the results
of Drakulic et al. (2005), the intracellular GSH concentration was higher during the planktonic exponential phase (from 1.6 mM in the gsh1 mutant to 11.2 mM in BY4774) than during the planktonic stationary phase (from 0.23 mM for gsh1 to 3.35 mM for BY4774).

In biofilms, the intracellular GSH concentration was very low for gsh1 (8.5 μM) and gsh2 (40 μM). In mats of the other strains, it ranged from 1.43 mM for glr1 to 5.35 mM for BY4774. Although most of the other deletion mutants growing in mats showed higher intracellular GSH concentrations than their planktonic-stationary-phase counterparts, the observed differences were not significant.

Because both gsh1 and gsh2 deletion mutants should be unable to synthesize GSH, the low intracellular level of GSH observed in these mutants probably came from the YPD medium, which contains about 0.5 mM GSH (Lee et al., 2001). In keeping with the role of GSH2 in glutathione biosynthesis, the gsh2 deletion mutant was found to accumulate γ-glucys under all growth conditions to a level comparable to that of GSH in the cis2, glr1, ycf1, opt1/hgt1, and yap1 strains. In all strains except the gsh2 deletion mutant, the intracellular γ-glucys concentration was low (Fig. 4b), remaining consistently below 0.44 mM in the stationary phase and even becoming undetectable in this phase in the gsh1, glr1, ycf1, and opt1/hgt1 strains. Its pattern of variation according to the state of development (planktonic exponential or stationary phase, 13-day-old mats) was different for different strains. For example, it increased in the reference strain from the planktonic exponential phase to biofilms but it decreased in that order in the gsh2 mutant. In other strains, it was maximal in mats, minimal during the stationary phase, and intermediate in the exponential phase.

The GSH concentration of about 11.2 mM observed in the reference strain BY4774 is comparable to the values of 8.0–12.4 mentioned by Drakulic et al. (2005) for the reference strains CY4 and JP0402. These investigators, furthermore, found exponentially growing CY4 Δyap1 and CY4 Δglr1 to exhibit intracellular GSH concentrations of 7.9 and 4.19 mM, respectively, similar to the values found in the corresponding deletion mutants investigated here (7.2 mM for BY4774 Δyap1 and 4.67 mM for BY4774 Δglr1). In the present work, the fact that intracellular GSH concentrations were lower in the ycf1 mutant than in the reference strain BY4774 might be due to feedback inhibition exerted by GSH on γ-glutamylcysteinyl ligase (GSH1) (Richman & Meister, 1975).

In mammalian cells, a high GSH content is often linked to cell proliferation (Shaw & Chou, 1986), whereas GSH depletion correlates with differentiation (Schafer & Buettner, 2001). In our case, there was a poor correlation, if any ($r^2 = 0.54$), between intracellular GSH concentration, cell proliferation, and budding. Because no significant differences were observed between mat-forming cells and planktonic-stationary-phase cells in terms of GSH content, intracellular GSH depletion does not emerge as a marker of cell differentiation, unlike the higher proportion of cells in the G1 state (Table 3).

Differences observed between strains might be due to interactions between the GSH-glutaredoxin and thioredoxin (TRX1 and TRX2) systems (Muller, 1991). Although this possibility remains to be evaluated, some tentative conclusions might be drawn in the light of this hypothesis. In all experiments, the yap1 strain showed a considerable proportion of cells in the S stage of the cell cycle (Table 3). Consistent with the fact that TRX2 is a primary target of Yap1p (Kuge et al., 1997), the yap1 strain showed a phenotype similar to that oftrx1trx2 mutants. In the stationary phase, the proportion of cells in the S phase was higher for all deletion mutants than for the reference strain, with the exception of the gsh1 and cis2 strains (Table 3). Thus, the absence of GSH1 and GGT in the planktonic stationary phase would appear to have an effect opposite to that of deletion of both thioredoxin genes, while deletion of the other tested genes seems to have the same effect as this double deletion. A putative thioredoxin activity

| Table 3. Proportion of cells (Saccharomyces cerevisiae reference strain for the reference strain and deletion mutants) in the G1, S, and G2/M phases of the cell cycle measured during the exponential and stationary phases of planktonic cells and in mats |
|-----------------|---|---|---|
|                  | G1 (%) | S (%) | G2/M (%) |
| Exponential phase |        |      |          |
| BY4774           | 37.6   | 18.3 | 44.1     |
| Δgsh1            | 28.4   | 57.2 | 14.4     |
| Δgsh2            | 28.5   | 42.2 | 33.8     |
| Δglr1            | 39.8   | 31.1 | 29.1     |
| Δycf1            | 47.1   | 33.5 | 19.4     |
| Δopt1/hgt1       | 31.1   | 35.8 | 33.1     |
| Δcis2            | 37.3   | 29.8 | 32.9     |
| Δyap1            | 21.2   | 49.3 | 29.5     |
| Stationary phase |        |      |          |
| BY4774           | 65.3   | 23.2 | 11.5     |
| Δgsh1            | 53.1   | 18.9 | 28.0     |
| Δgsh2            | 50.9   | 29.3 | 19.9     |
| Δglr1            | 67.3   | 30.7 | 2.0      |
| Δycf1            | 46.7   | 27.3 | 26.0     |
| Δopt1/hgt1       | 46.8   | 29.9 | 23.3     |
| Δcis2            | 53.3   | 18.9 | 28.0     |
| Δyap1            | 22.0   | 33.5 | 44.4     |
| Mats             |        |      |          |
| BY4774           | 70.7   | 17.6 | 11.7     |
| Δgsh1            | 59.6   | 18.2 | 29.3     |
| Δgsh2            | 60.6   | 24.8 | 14.6     |
| Δglr1            | 62.5   | 2.1  | 35.4     |
| Δycf1            | 64.0   | 20.2 | 15.8     |
| Δopt1/hgt1       | 67.0   | 19.9 | 13.1     |
| Δcis2            | 59.0   | 31.1 | 9.9      |
| Δyap1            | 55.2   | 26.6 | 18.2     |

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enhancement in gsh1 and cis2 strains could be linked to an overlap between the roles over thioredoxins and the glutathione–glutaredoxin system. In biofilm-forming cells, the proportion of cells in the S phase was higher in all strains than in the reference strain, with one exception: the glr1 strain. Hence in mat-forming cells, deletion of genes involved in GSH reduction appears to have an effect opposite to that of TRX deletions (Muller, 1991).

Effects of gene deletion and physiological state on hydrogen peroxide tolerance

The different tested strains showed somewhat lower tolerance to H2O2 during the exponential phase than during the stationary phase. Tolerance was maximal in biofilms (Fig. 5a), possibly because growth was slow. Expression of both thioredoxin genes increases in the stationary phase, leading to better resistance to hydroperoxides (Garrido & Grant, 2002). This might explain the differences observed here between the exponential and the stationary phases.

In this experiment, the gsh1 strain surprisingly showed a tolerance towards H2O2 similar to that of the reference strain, and far greater tolerance than glr1. It seems that GSH protection against oxidative stress depends on the presence of a functional glutathione reductase (GLR1), the key regulatory enzyme determining the redox state of the GSH–glutaredoxin system. Elskens & Penninckx (1997) have shown that glutathione reductase activity is essential in S. cerevisiae for tolerance to the oxidant fungicide thiram. Muller (1996) has shown that loss of glutathione reductase leads to accumulation of oxidized glutathione, making thioredoxin necessary for growth. The yap1 strain displayed the lowest tolerance to H2O2. YAP1p is a basic-leucine zipper transcription factor that controls, among others, the gsh1, trx1, trx2, and ycf1 genes; trx2 is a major H2O2 stress tolerance factor (Garrido & Grant, 2002).

An examination of the above H2O2 tolerance data in the light of the measured intracellular GSH concentrations leads to the conclusion that the GSH level may be an important factor, but not the only one, determining the level of H2O2 tolerance. For example, cells in the exponential phase exhibited higher intracellular GSH concentrations (Fig. 4a) but lower tolerance to H2O2 than stationary-phase cells (Fig. 5a). There are conflicting results regarding whether GSH plays any role in the adaptive response of cells to H2O2 (Izawa et al., 1995; Grant et al., 1996; Stephen & Jamieson, 1996; Lee et al., 2001), but there is agreement that reference levels of GSH are not required to elicit an adaptive response to H2O2 stress (Davies et al., 1995; Lee et al., 2001).

The data on H2O2 tolerance in relation to the gene deleted in each strain highlight the importance of glutathione reductase. It is noteworthy that the relatively high pool of GSH in exponentially growing glr1 cells affords less protection against H2O2 than the very low GSH pools
present in the gsh1 and gsh2 mutants (Fig. 5a). Hence, any protection provided by GSH must depend on glutathione reductase. The H₂O₂ tolerance of the Δgsh1 mutant might possibly be due to enhanced TRX activity taking over from the glutaredoxin system. Operation of the GSSG/2GSH couple might thus be less crucial than TRX2 in adapting to oxidative stress (Trotter & Grant, 2003). The TRX proteins do seem essential for the response of *S. cerevisiae* to oxidative stress induced by hydroperoxides (Garrido & Grant, 2002). The results obtained with the yap1 mutant further support the view that TRX2 plays a major role in hydroperoxide tolerance, because TRX2 is the primary target of Yap1p (Kuge et al., 1997) and because the yap1 deletion mutant shows by far the lowest tolerance to H₂O₂.

As seen in Fig. 5a, H₂O₂ tolerance was the highest in biofilm-forming cells of all the strains studied, lower in planktonic stationary-phase cells and the lowest in planktonic exponential-phase cells. That stress tolerance in growing cultures is related to the position of individual cells in the cell cycle has also been reported for heat resistance in *S. cerevisiae* (Plesset et al., 1987). Leroy et al. (2001) report that the high sensitivity to H₂O₂ observed during the S phase could be the result of ineffective DNA base excision repair. We observed a negative correlation between H₂O₂ tolerance and the proportion of cells in the S phase of the cell cycle. Thus, the high H₂O₂ tolerance of biofilm-forming cells may be linked to the higher proportion of G1 cells as compared with S cells.

### Effects of gene deletion and physiological state on Cd²⁺ tolerance

Cadmium is among the nonessential heavy metals that are toxic and probably carcinogenic at low concentrations. Cd²⁺ toxicity depends on its ability to form complexes with biological antioxidant compounds (Stohs & Bagchi, 1995), thus causing oxidative stress. We therefore investigated cadmium tolerance at a high concentration (10 mM) in *S. cerevisiae* strains impaired in GSH metabolism and the oxidative stress response. Figure 5b shows unambiguously that cadmium tolerance was higher in the stationary phase than in the exponential phase and also higher in biofilm-forming cells than in stationary-phase planktonic cells. The results are similar to the data obtained with H₂O₂ tolerance (Fig. 5a) and could also be a consequence of the distribution of the corresponding populations among the different phases of the cell cycle. In a flow cytometry study of copper sensitivity in asynchronously grown *S. cerevisiae*, sensitivity was linked to the cell-cycle stage (Howlett & Avery, 1999). Sensitivity has also been linked to age-dependent activation of Sod1p (Sumner et al., 2003).

The strain-to-strain differences observed here provide information on genetic determinants of cadmium tolerance. The most Cd²⁺-sensitive strains are glr1, ycf1, cis2, yap1, and to a lesser extent gsh1 (Fig. 5b). The yeast cadmium factor YCF1 mediates accumulation of Cd–GSH complexes in the vacuole, which is an efficient detoxification pathway.
H2O2 and Cd2+ displayed major differences in tolerance to stress caused by the cell cycle. Deletion of vacuolar vacuolar accumulation of the bis–glutathionato–cadmium complex Cd–GSH2. Deletion of vacuolar γ-glutamyl transpeptidase (cis2) has an effect similar to that of ycf1 deletion. This reflects the fact that γ-glutamyl transpeptidase activates the YCF1 system (Mehdi et al., 2001). Mediation of Cd2+ transport by GSH depends on the presence of a functional glutathione reductase. The cadmium sensitivity of the gir1 strain (Fig. 5b) may be an effect of GSSG accumulation and inefficient GSH turnover. The dipeptide γ-glucys might be able to form a complex with cadmium, as shown by the tolerance of the gsh2 strain. A complex formed between GSH and the dipeptide might possibly serve as a substrate for YCF1 in the absence of GSH. In the absence of both γ-glucys and GSH (gsh1 deletion mutant), Cd2+ would accumulate in the yeast cytoplasm, leading to oxidative stress. The moderate tolerance of the gsh1 deletion mutant seems more difficult to explain; it might be due to activation of the TRX genes, which might limit oxidative-stress-induced damage.

In addition to the cell-cycle phase, yeast cell age should be taken into account as an important factor affecting stress sensitivity (Drakulic et al., 2005). Cell age is expressed as a number of generations, because S. cerevisiae cells divide a finite number of times before senescence. In S. cerevisiae, oxidatively damaged proteins are preferentially retained in mother cells rather than in daughter cells; this contributes to the deterioration of mother cells and to the fitness of daughter cells (Aguilaniu et al., 2003).

Saccharomyces cerevisiae biofilms and planktonic cells displayed major differences in tolerance to stress caused by H2O2 and Cd2+. In bacteria, the higher stress resistance of biofilm-included cells as compared with planktonic cells involves various sigma factors (van Schaik & Abee, 2005), quorum-sensing molecules (Nalca et al., 2006), and exopolysaccharides (Wai et al., 1998). We conclude here that both cell-cycle distribution and cell age are likely to be important determinants of resistance to H2O2 and Cd2+ in the case of fungal biofilms, in particular, those formed by S. cerevisiae. Biofilm-forming cells might also provide a good model for studying interactions between GSH glutaredoxin and thioredoxin systems in S. cerevisiae and their relationship with the cell cycle.

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