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# Comparing the rate of growth and metabolic efficiency of yeast experiencing environmental stress or genetic damage*

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**Physical stresses, toxic substances, and mutations can cause marked decline in the rate of growth (RG). We report that the efficiency of growth (EG), i.e. converting glucose into biomass, responds less profoundly. It remains nearly unaffected for some physical and chemical stresses, but decreases substantially for others, specifically those affecting membrane integrity or ion homeostasis. Mutations (gene deletions) can heavily reduce RG, but much less EG. Moreover, there is no apparent relation between the function of deleted gene and EG. Generally, assays of EG appear as more laborious, less precise, and less informative than those of RG.**

**Key words**: fitness cost, growth rate, metabolic efficiency, environmental stress, chemical stress, gene deletion

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## INTRODUCTION

The construction of a gene-deletion collection allowed to test the impact of hundreds of physical and chemical stress factors on thousands of yeast strains (Giaever *et al.*, 2002, Steinmetz *et al.*, 2002, Hillenmeyer *et al.*, 2008, Blomberg 2011). All these studies were based on the assumption, that malfunctioning of a deletion strain in a particular environment is a sign of the deleted gene's normal usage in it. Although specific experimental arrangements differ, most studies rely on one phenotypic trait, the rate of growth (RG). Efficiency of growth (EG), defined as the efficiency of converting resources into biomass, could be another one. There were few attempts to estimate EG for the whole collection of yeast gene deletions. In those studies, however, neither the production of biomass nor consumption of glucose were actually measured. Instead, only the amount of biomass was estimated, and not directly but through measuring the optical density (OD) of cultures in stationary phase. One study of this type found such data much less useful than those relating to RG (Warringer *et al.*, 2003), while another research suggested, that they can be informative (Bell, 2010). In both these studies, however, actual amount of consumed resources was unknown. This is because even if all deletion strains consumed glucose completely, fast-growing strains had more time to metabolize ethanol (and thus add biomass) than slow-growers before OD was measured. Moreover, OD is only an indirect estimate of biomass and is sensitive to the morphology of cells, for example, their size. The yeast deletion strains differ substantially in cell morphology, and therefore, comparison of OD of different strains can be misleading (Jorgensen *et al.*, 2002).

We consider it more appropriate to work with cells growing exponentially in glucose-rich media. This stands to reason that in such media glucose is a sole source of energy, because even though ethanol accumulates, it is not metabolized due to strong allosteric and transcriptional repression of oxidative metabolism (Zaman *et al.*, 2008). Metabolic/energetic expenses of such cells can be accurately approximated by measuring the rate of glucose uptake. A reliable method of estimating the yield of biomass is to weigh dry mass of cells. However, assays of glucose consumption and dry mass production are laborious,. They were not done for the whole collection of gene-deletions. In our recent study of epistasis, we have got estimates of the biomass production per consumed glucose for a sample of strains with a different number of gene deletions (Jakubowska & Korona, 2012). The estimates of biomass were somewhat improved when compared with previous studies, but still imperfect. We have found that strains with single gene deletions differ in EG, but the differences are probably modest. In the work described here, we use a sample of deletion strains to estimate both RG and EG possibly most accurately. We also asked how RG and EG change when not deletions of genes but external stresses are present. In this ways, we wanted to test how the changes in RG and EG caused by mutations compare to those imposed by unfavorable environmental conditions.

## MATERIALS AND METHODS

**Strains and media.** The strain used to test the impact of chemical and physical factors was *BY4742 MATa leu2 his3 ura3 lys2*. It is the strain in which complete collection of yeast gene deletions was engineered (Giaever *et al.*, 2002).

Growth media consisted of YPD (1% yeast extract, 2% peptone, 2% glucose) supplemented with chemicals at defined concentrations. Test media were obtained by mixing double-concentrated YPD with equal aliquots of water with fresh solutions of test chemicals prepared at concentrations twice higher than final ones. Stock solutions were kept in water or, in case of nystatin and benomyl, in DMSO. The agents and their modes of action are listed in Table 1. Specific concentrations used in this study were as follows: benomyl (μg/ml) 15, 28,

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**Abbreviations**: RG, rate of growth; EG, efficiency of growth; OD, optical density

40; caffeine (mg/ml) 0.80, 1.35, 2.50; cold (°C) 25, 22, 18; $CuSO_4$ (mg/ml) 2.0, 2.5, 2.8; cycloheximide (ng/ml) 20, 80, 120; DMSO (%) 5.0, 7.5, 8.0; ethidium bromide (μg/ml) 30, 55, 85; KCl (mg/ml) 70, 120, 140; methanol (%) 5.0, 7.0, 8.5; NaCl (mg/ml) 35, 60, 80; paromomycin (mg/ml) 0.2, 0.4, 0.9; $ZnSO_4$ (mg/ml) 0.65, 1.00, 1.50. As explained in Results, some factors were tested at one level only: benzoic acid (mg/ml) 1.0; diamide (mg/ml) 0.25; EDTA (mg/ml) 0.1; high pH (pH) 9; hygromycin B (μg/ml) 20; nalidixic acid (mg/ml) 0.5; nystatin (μg/ml) 1.4; SDS (mg/ml) 0.35; sodium salicylate (mg/ml) 25; sorbitol (mg/ml) 380.

**Gene deletions.** A sample of about two hundred strains was tested for the efficiency of converting glucose into biomass in our former study (Jakubowska, Korona, 2012). Those measurements relied on OD scores corrected for the size of cells only, and therefore, are indirect estimates of biomass only. Here, we carried out direct measurements of growth rate, glucose consumption, and dry mass for a sample of 20 gene deletions. The 20 gene deletions were selected as those promising to show substantial decrease in EG. The deleted genes included: *TPM1,* actin assembly; *GND1,* response to oxidative stress; *IMG2,* mitochondrial translation; *ELP3,* transcription; *LRP1,* RNA metabolism; *MRH4,* ribosome biogenesis; *TAF14,* chromatin remodeling; *RAD54,* DNA translocase; *VID22,* protein catabolism; *DSS1,* RNA metabolism; *COQ3,* ubiquinone synthesis; *RAD6,* DNA ligase; *BUB1,* cell cycle; *AIM10,* proline-tRNA ligase; *RPL8A,* translation; *MET7,* methionine biosynthesis; *REG1,* carbohydrate metabolism; *SSN8,* transcription; *PMR1,* calcium transport ATPase; *HUR1,* DNA replication. Functional descriptions are borrowed from the Saccharomyces Genome Database (www.yeastgenome.org).

**Growth rate, glucose consumption, dry mass.** Experimental cultures were initiated by inoculating 25 μl of overnight culture into 10 ml of fresh medium. The new cultures were kept in vials of 30 mm in diameter, loosely capped, and agitated at 250 rpm. Temperature was set to 30°C except for the cold stress gradient. After reaching $OD_{600}$ of about 0.1, aliquots of 200 μl were taken every 1 h and their density was measured. Because OD readings showed a very good fit to the exponential growth curve for ODs between 0.1 and 0.4, OD scores within this interval were log-normal transformed, and used to calculate the rate of growth (RG).

Growth of the cultures was terminated when $OD_{600}$ was between 0.4 and 0.5 by transferring the vials to ice water and vigorous shaking. A sample of 5 ml was taken from every culture and spun down; pellet was preserved for further assays of dry mass; a sample of supernatant was saved to assay glucose concentration. The latter was done by diluting supernatant 1:200 and applying a kit based on an enzymatic reaction and fluorescence reading (D-GLUCOSE-HK, Megazyme). Pellets obtained from the 5 ml samples were washed three times, vacuum dried, and then left loosely capped for 5 days in a drier set to 65°C. The final weight of the dried pellets was a few milligrams, they were weighed with an accuracy of one hundred of a milligram.

All measurements were replicated three times, that is, three independent cultures were initiated and their maximum growth rate, glucose consumption, and dry mass production were individually estimated. One replica of the diamide treatment was lost.

## RESULTS

### Environmental stresses

We started with 12 chemical or physical agents for which we were able to obtain incremental deceleration of growth by about 1/4, 1/2, and 3/4. Doses required for these defined effects were found in pilot experiments. Figure 1 shows what were the effects of specific agents on RG and EG. A quadratic regression line was fitted in every case to show a general trend. Gradients of stress could not be completed for 10 agents. A chief rea-

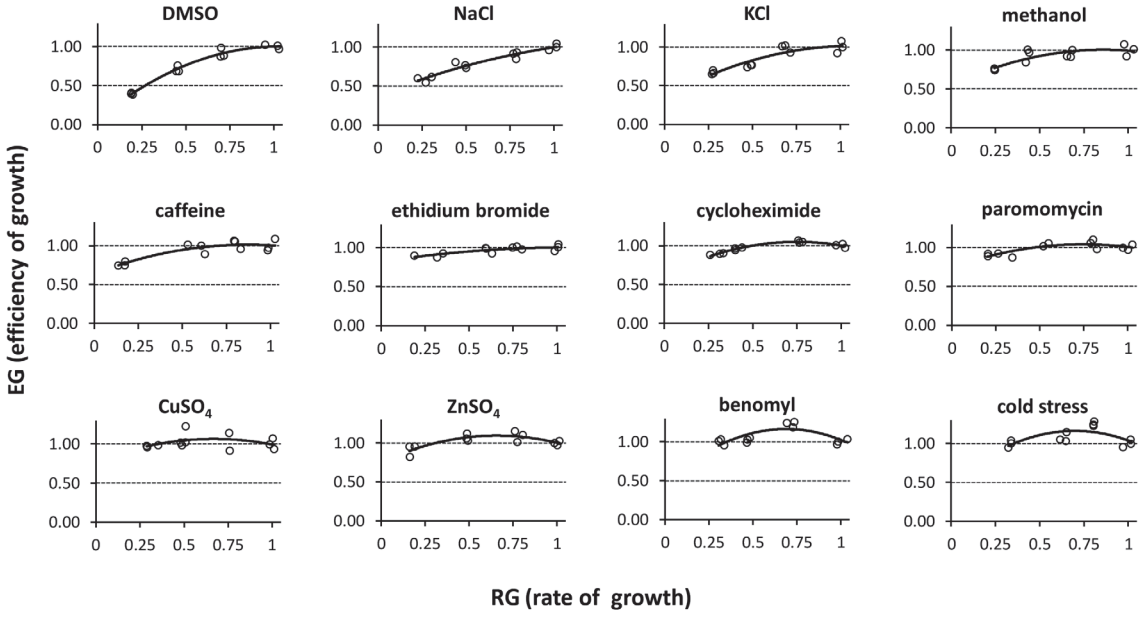


**Figure 1. Efficiency of growth (EG) along stress gradients.**
Horizontal axes: normalized rate of growth (RG). Vertical axes: normalized EG, that is the ratio of dry mass over consumed glucose. Both variables were normalized by dividing them respectively by average RG and EG in non-stressful environment. The doses of stress factors reducing RG to 0.75, 0.5 and 0.25 are listed in Materials and Methods.

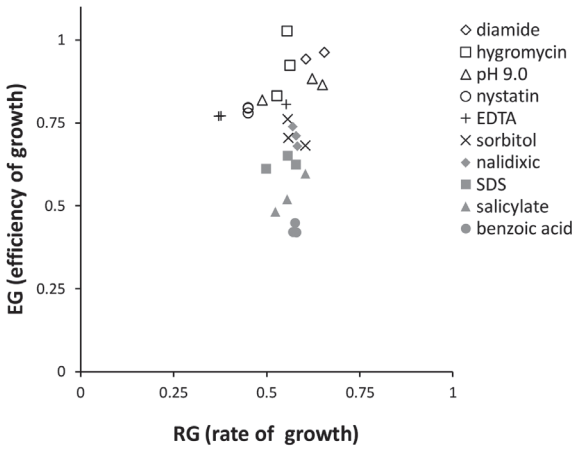


**Figure 2. Efficiency of growth (EG) under single-intensity stresses.**
Axes and normalization as described in Fig. 1.

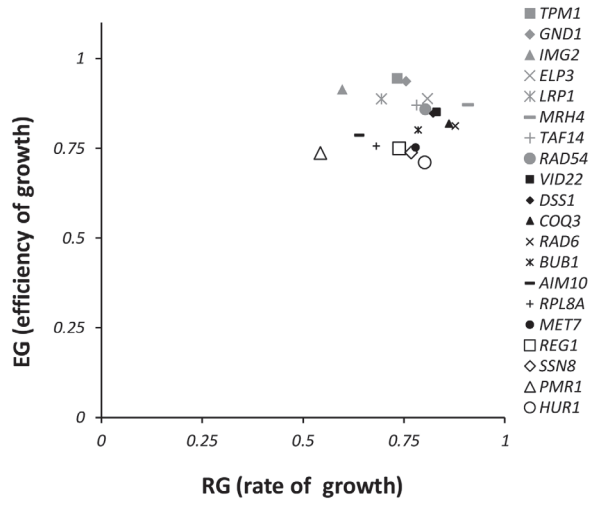


**Figure 3. Efficiency of growth (EG) of gene deletions strains.**
Means instead of three individual estimates per gene deletion are shown. Axes as described in Fig. 1. Normalization was done by dividing RG and EG of deletion strains by RG and EG of the strain in which deletions were done. RG and EG were measured in non-stressful environment. Gene functions are listed in Materials and Methods.

son was a precipitous decrease in RG at narrow regions of stress intensities. Moreover, the critical doses of stress factors were apparently very much affected by even slight fluctuations in the preparation of stocks, working media, and conditions of culturing. In particular, growth curves became irregular making calculation of RG unre-

**Table 1. Stress agents.**

| Agent | Mode of action | Reference |
|---|---|---|
| Benomyl | cytoskeleton depolimerization | (Hoyt *et al.*, 1990) |
| benzoic acid | destabilization of membrane proton gradient | (Verduyn *et al.*, 1992) |
| Caffeine | disturbance of growth signaling pathways | (Wanke *et al.*, 2008) |
| cold | ineffective assembly of protein complexes | (Guthrie *et al.*, 1969) |
| $CuSO_4$ | oxidative stress | (Yasokawa *et al.*, 2008) |
| cycloheximide | inhibition of protein synthesis | (Stocklein Piepersberg, 1980) |
| Diamide | oxidative stress | (Wemmie *et al.*, 1997) |
| DMSO | decreased cell membrane integrity | (Murata *et al.*, 2003) |
| EDTA | increased cell wall porosity | (de Nobel, 1989) |
| ethidium bromide | inhibition of DNA and RNA polimerization | (Newton, 1957) |
| high pH | alkaline stress, ionic imbalance | (Serrano, *et al.*, 2004) |
| hygromycin B | interference with translation elongation | (Chuang & Madura, 2005) |
| KCl | osmotic stress | (Arino *et al.*, 2010) |
| Metanol | destabilization of cell membrane structure | (Yasokawa *et al.*, 2010) |
| NaCl | osmotic stress, K+/Na+ imbalance | (Arino *et al.*, 2010) |
| nalidixic acid | inhibition of topoisomerase | (Ludewig *et al.*, 1994) |
| Nystatin | increased cell membrane parmeability | (Venables & Russell, 1975) |
| paromomycin | decreased fidelity of translation | (Balasundaram *et al.*, 1999) |
| SDS | damaged cell membrane | (Sirisattha, *et al.*, 2004) |
| sodium salicylate | acidification of cytoplasm | (Giardina Lis, 1995) |
| Sorbitol | osmotic stress | (Hirasawa *et al.*, 2006) |
| $ZnSO_4$ | interference with ion-binding proteins | (Pagani *et al.*, 2007) |

liable. For these stresses, we decided to concentrate on single doses, which would be intense enough to cause about 50% reduction of RG. Fig. 2 shows RGs and EGs for these agents.

Figures 1 and 2 show that EG varied depending on what factor was tested. This is especially well visible in Fig. 1, where some stress factors nearly do not change EG even though RG is four times lower than under no-stress conditions, while other stresses lead to pronounced decreas in EG. In case of the single-intensity stresses (Fig. 2) the reaction of EG at the 0.5 RG was on average stronger than that in the gradients. In sum, the two used measures of stress intensity, RG and EG, can give considerably contrasting patterns of reaction to eternal stress. Under most conditions tested here, RG was sensitive and monotonic in its reaction to increasing stress intensity. The reaction of EG was neither equally universal nor comparably strong.

### Gene deletions

Figure 3 shows EGs and RGs for 20 gene deletions. None of the deletions reduced EG as much as the most damaging stresses shown in Figs. 1 and 2. Reduction in RG was also less pronounced than under the most severe eternal stresses. It was already noted, however, that the 20 deletions were chosen from a sample of about 200 strains as those likely to have especially large negative effects on EG, not RG.

## DISCUSSION

Our experiments demonstrate that two measures of reaction to stress, RG and EG, can give considerably different results. Under most environmental conditions tested here, RG declined strongly as the intensity of stress increased. The efficiency of converting glucose into biomass was not equally sensitive. Under some stresses EG decreased significantly, but there were also stresses in which hardly any change was seen. Comparison of Figs. 1 and 2 with Table 1 leads to a conclusion, that the most damaging to EG were those factors, which affected membrane gradients, or caused strong osmotic stresses. Intense rebuilding of the gradients and deep restructuring of the cell metabolism under high osmotic pressure are known to require large amounts of energy (Hohmann, 2002). Factors that did not affect EG, or did it only marginally, were more diverse. Some were very specific or had narrow targets. For example, aminoglycoside antibiotics slow down the process of translation; however, they do not destroy its key and costly machinery, namely, the ribosome subunits. Other, as divalent metal ions, tend to outcompete other ions in binding specific proteins, but in this experiment their concentration was generally low, and thus they did not cause the osmotic stress or strong ionic disequilibrium.

Regarding mutational damage, our present assays confirm earlier observations, that mutations can cause a loss in EG. The sample of gene deletions used in this study was not random, but chosen as those expected to result in an especially strong negative impact on EG. But, even though these were the deletions most damaging to EG, their absolute effects were rather moderate when compared with those accompanying external stresses. The sample size was rather small, but the tested genes were substantially different from each other in terms of functions they coded for (Materials and Methods). Unlike stresses, which turned out to be especially damaging when membrane integrity was affected, the gene deletion with highest EG effects had no apparent common (prevailing) function. This suggests that low EG is only one of multiple phenotypes affected by deletion of these pleiotropic genes, and therefore its applicability to functional analyses may be limited. This finding, and the fact that the effects of gene deletions on EG were rather low, questions the rationale for using EG estimates for phenotypic assays of large collections of mutants. The reason is that measurements of EG are both much more laborious and less precise than that of RG. A question arises, how much information is lost or misinterpreted when EG is not assayed. This could be important for the assays in which the rate of growth of multiple strains is measured simultaneously on a common agar plate. The eventual differences in the colony size are affected both by RG and EG, but it is common to ignore the impact of EG (Blomberg, 2011). We think that this practice is likely allowable because EG can have any impact only when colonies are large enough to use non-trivial share of glucose present in agar. Furthermore, as our results suggest, even when the differences in EG are present, their final effect would be relatively weak.

In our earlier study, we have pointed out that some mutations can lead to a substantial decrease in the rate of growth, but not in the metabolic efficiency (Jakubowska & Korona, 2012). This was confirmed in the present study (Fig. 3). However, although there was no general tendency that the metabolic efficiency declined with the rate of growth, there were mutations which were evidently deleterious to the efficiency. Considering environmental stresses, their effects on the efficiency were similarly different: some environments had a clearly negative impact while others appeared completely neutral. The impact of stresses on the efficiency is partly understood, at least for some environments (Hohmann, 2002). The question why only some mutations make the metabolism inefficient is well worth asking and can potentially lead to deeper understanding of the cell physiology.

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