

## Elevated Growth of *Saccharomyces cerevisiae* *ATH1* Null Mutants on Glucose Is an Artifact of Nonmatching Auxotrophies of Mutant and Reference Strains†

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**Yeast strains disrupted for *ATH1*, which encodes vacuolar acid trehalase, have been reported to grow to higher cell densities than reference strains. We showed that the increase in cell density is due to the *URA3* gene introduced as a part of the disruption and concluded that the misinterpretation is a result of not using a control strain with matching auxotrophic markers.**

The *ATH1* gene of *Saccharomyces cerevisiae* codes for vacuolar acid trehalase (1). Kim et al. (2) reported that disruption of this gene resulted in an almost twofold increase in the cell density attained in a yeast extract- and peptone-based medium with a high glucose concentration. These authors used *URA3* as a marker gene for disruption of *ATH1* and compared the *ura3 ATH1* parent strain (uracil auxotroph but wild type for *ATH1*) with the *ath1::URA3* disruptant (uracil prototroph but disrupted for *ATH1*). To separate the effects of uracil prototrophy and *ATH1* disruption, we deleted *ATH1* in a prototrophic strain (FY4) isogenic to S288C (9) by using a kanMX module as the selectable marker (8). Deletion of *ATH1* was confirmed by PCR and by the inability of the mutant (DA1) to utilize trehalose as a sole carbon source (3). Growth of the strains was compared in YP medium (1% Bacto Yeast Extract [Difco], 2% Bacto Peptone [Difco]) containing 25% glucose. The cell density attained by DA1 was comparable to the cell density of FY4, which indicated that the  $\Delta$ *ath1* mutation did not affect growth under these conditions, and was greater than the cell density of FY3, an isogenic *ura3-52* mutant of FY4 (9). Growth of RC47, a *Ura*<sup>+</sup> transformant of FY3, was comparable to growth of FY4, confirming that the poor growth of FY3 was due to uracil auxotrophy. When the medium was supplemented with 40 mg of uracil per liter, the strain FY3 cell density was the same as the cell density of the *Ura*<sup>+</sup> strains (Fig. 1). Kim et al. (2) compared the growth of the *ATH1* parent strain (SEY6210), a uracil auxotroph, with the growth of the  $\Delta$ *ath1::URA3* mutant (MDY3), a uracil prototroph. Our results suggest that the increased growth which Kim et al. attributed to the  $\Delta$ *ath1* mutation is due to the uracil prototrophy of the  $\Delta$ *ath1* mutant and that differences in growth should disappear when the uracil limitation is relieved.

To study the effect of disrupting a gene, ideally the disruptant should differ from the control strain only with respect to the disrupted gene. However, in many gene disruption studies the disruptant and control strains differ in two respects, namely, in the disrupted gene and in the auxotrophic marker used for the disruption. Many researchers assume that the auxotrophic marker is phenotypically neutral, which may often

be true. However, if the auxotrophic supplement is limiting, the absence of control strains with matching auxotrophy can result in significant errors. The fact that uracil is present in a limiting amount in YP medium is not well appreciated; the reason for this may be the fact that in standard YPD medium containing 2% glucose the cell densities of the *Ura*<sup>+</sup> and *Ura*<sup>-</sup> strains may not be strikingly different. For example, the final cell density of FY3 in YPD medium was about 80% of the final cell density of FY4. When the medium was supplemented with additional uracil, FY3 grew to the same cell density as FY4, implying that in standard YPD medium cells may reach the stationary phase due to starvation for uracil or glucose, depending on the auxotrophy of the strain.

Even if uracil is present in the medium, under some conditions the cells are not necessarily able to take it up (6). Star-

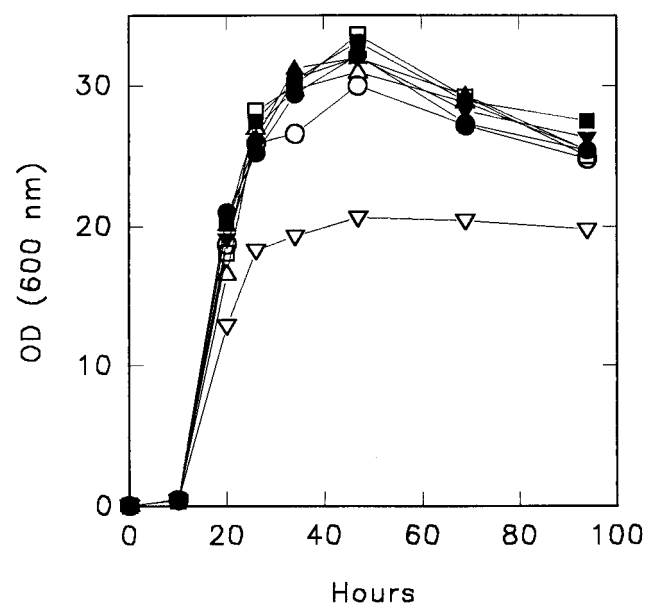


FIG. 1. Growth of *S. cerevisiae* strains in YP medium containing 25% glucose without (open symbols) or with (solid symbols) additional uracil (40 mg/liter) at 30°C. The following strains were tested: FY4 (○) and RC47 (□), which are prototrophic for uracil and wild type for *ATH1* (*URA3 ATH1*); DA1 (△), which is prototrophic for uracil and deleted in *ATH1* (*URA3 Δath1::kanMX*); and FY3 (▽), which is auxotrophic for uracil and wild type for *ATH1* (*ura3-52 ATH1*). OD (600 nm), optical density at 600 nm.

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vation for a nitrogen, phosphate, or carbon source can trigger rapid degradation of uracil permease and a loss of uracil uptake (7). Thus, the physiological results may be different for uracil auxotrophs and prototrophs, even if the medium is supplemented with sufficient uracil. Similarly, auxotrophic markers involved in amino acid biosynthesis are considered ill-suited for disrupting genes in intermediary carbon metabolism, as amino acid biosynthesis is an integral part of this metabolism (5). We suggest that markers such as the kanMX module conferring G418 resistance (8) should be used as alternatives to auxotrophic markers in gene disruption studies (4).

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#### REFERENCES

1. **Destruelle, M., H. Holzer, and D. J. Klionsky.** 1995. Isolation and characterization of a novel yeast gene, *ATH1*, that is required for vacuolar acid trehalase activity. *Yeast* **11**:1015–1025.
2. **Kim, J., P. Alizadeh, T. Harding, A. Hefner-Gravink, and D. J. Klionsky.** 1996. Disruption of the yeast *ATH1* gene confers better survival after dehydration, freezing, and ethanol shock: potential commercial applications. *Appl. Environ. Microbiol.* **62**:1563–1569.
3. **Nwaka, S., B. Mechler, and H. Holzer.** 1996. Deletion of the *ATH1* gene in *Saccharomyces cerevisiae* prevents growth on trehalose. *FEBS Lett.* **386**:235–238.
4. **Oliver, S.** 1996. A network approach to the systematic analysis of yeast gene function. *Trends Genet.* **12**:241–242.
5. **Pronk, J. T., H. Y. Steensma, and J. P. van Dijken.** 1996. Pyruvate metabolism in *Saccharomyces cerevisiae*. *Yeast* **12**:1607–1633.
6. **Varela, J. C. S., C. van Beekvelt, R. J. Planta, and W. H. Mager.** 1992. Osmostress-induced changes in gene expression. *Mol. Microbiol.* **6**:2183–2190.
7. **Volland, C., D. Urban-Grimal, G. Geraud, and R. Haguenaer-Tsapis.** 1994. Endocytosis and degradation of the yeast uracil permease under adverse conditions. *J. Biol. Chem.* **269**:9833–9841.
8. **Wach, A., A. Brachat, R. Pohlmann, and P. Philippsen.** 1994. New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* **10**:1793–1808.
9. **Winston, F., C. Dollard, and S. L. Ricupero-Hovasse.** 1995. Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. *Yeast* **11**:53–55.