

Identification of a putative amidase gene in yeast *Saccharomyces cerevisiae*

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We have identified a gene in yeast *Saccharomyces cerevisiae* sharing significant similarities with the *Aspergillus nidulans amdS* gene (57.2% similarity and 33.2% identity) and to the *Pseudomonas syringae iaaH* gene (47.6% similarity and 22.2% identity). The *amdS* and *iaaH* genes code for the enzymes acetamidase and indoacetamide hydrolyase, respectively (1, 2). The sequence identities between *amdS* and the putative yeast amidase gene are underlined as shown. The putative amidase gene was discovered during characterization of the putative yeast RNA helicase gene, *CA8* (3). These two genes are arranged in a tail-to-tail fashion and are separated by 61 nucleotides. The putative yeast amidase gene can potentially code for a gene product of 549 amino acids in length.

To determine whether this gene is essential, we disrupted the cloned gene by replacing an internal MluI-AatII fragment with an auxotrophic marker *URA3*. This disrupted gene was used to transform a ura$^-$ diploid strain for replacement of one of the two wild-type putative amidase genes through homologous recombination. Gene replacement in the ura$^+$ transformants was subsequently verified by Southern blot analysis. A strain heterozygous (one wild-type copy and one *URA3*-disrupted copy) at the putative amidase gene locus gave rise to four viable spores upon sporulation. The ura3$^+$ knock-out spores displayed no phenotypic difference from the wild-type ura3$^-$ spores when grown on several different media (YPD, YP/glycerol, and YPD/1.5 M NaCl) at various temperatures, and were able to mate normally. In addition, the sporulation efficiency of the doubly-disrupted strain appeared to be similar to that of the heterozygous and the wild-type strains. Taken together, these data suggest that this putative amidase gene is not essential for either vegetative or meiotic growth in yeast cultured under laboratory conditions.

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