

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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-100          -80          -60          -40          -20          1          20
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V Q L K K D Q L N S K I K D E W K L N S T T I T R L K N D K K N L I K N I D D L C S S S E N Q I T H
200         220         240         260         280         300         320
TCCACGATTAATGGCTTGAAGACAGGCACTGGAAGCAAAAGAGTTAAGCTGCCATGAAATAACAGCTGCATTTTGTGATAGGGTGGCTTAAATTCATCAAGTAGTGAATTTGCTATCCGAAATCATGTTTCAGAGGCAATTGAGATTGGCT
S T I M A L R Q A L E A K E L S C H E I T A A F C H R A A L I H Q V V N C L S E I M F S E A L R L A
340         360         380         400         420         440         460         480
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D Y Y D S N R P A I L P F L Y G I P I S L K D Q C N Y E G V D T S L G Y L C R T F K P K T K N E S
500         520         540         560         580         600         620
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L I V S F L R D L G A I I F V K T T V P S S M M A T D T Q S N T F G Y T Y N S I N L S F S S G G S S
640         660         680         700         720         740         760         780
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G G E G S L I G A H G S L L G L G T D I G G S I R I E S S Y Q G L F G L K E T F G R V P Y L R V D N
800         820         840         860         880         900         920
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S F E G R E T I P S V I G P L A R D L S D L R Y E M S C V I N I C Q P W V Q D V K C I P Y H F D S S
940         960         980         1000        1020        1040        1060        1080
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T S K L H D N Y V V G I W Y G D G V I D D P P S D I R A L K T C E D L V N K T K G M K A V K W E E S
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1400        1420        1440        1460        1480        1500        1520
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1540        1560        1580        1600        1620        1640
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N D L I Q S G E I D G F E I S L Q V V S P T F N D N E V C K F A S W L F S K I *

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