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THE GENETIC CHARACTERISTICS *SACCHAROMYCES CEREVISIAE* ACI⁺ MUTANTS

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Abstract: A series of 30 *Saccharomyces cerevisiae* aci⁺ mutants (characterized as acidifying Ogur's glucose medium containing bromocresol purple) were isolated after EMS mutagenesis. All the mutants excreted acid metabolites to the medium after 24 or 48 hours of incubation. The character of the aci⁺ mutations was defined using classical genetic techniques. Three of the aci⁺ mutants were studied by molecular genetics techniques.

Key Words: *Saccharomyces cerevisiae*, Yeast, TCA, aci⁺ Mutants

INTRODUCTION

Saccharomyces cerevisiae is a model organism in the analysis of eukaryotic genomes. A general advantage of yeast in such studies is that it is possible to use both classic and molecular genetics methods on it. The yeast genome sequence has been fully mapped out however, the functions of many of the genes have still not been explained, and the role of many ORFs has yet to be identified [1].

A series of 30 *Saccharomyces cerevisiae* mutants, marked as aci⁺, were characterized as acidifying Ogur's glucose medium supplemented with bromocresol purple as a pH indicator. Mutants extruding acidic metabolites produce colonies with a yellow zone on violet CK medium [2-4]. The first aci⁺ mutants were obtained on CK medium by Ogur *et al.* [5, 6]. Two mutants were isolated and described as *glt₁* and *glt₂*. They were deficient in mitochondrial aconitase, an enzyme of the Tricarboxylic Acid Cycle (TCA).

In the years following this pioneering work, yeast mutants with damage to their TCA cycle were isolated and the appropriate genes were cloned, sequenced and located [7-16]. So far, 15 different nuclear genes of *Saccharomyces cerevisiae*

have been described. The genes encode the protein subunits of 8 enzymes of the TCA cycle [17]. However, damage to TCA cycle genes is not the sole cause of extracellular acidification in yeast. Three novel genes complementing one of the *aci*⁺ mutations were isolated [18]. Those genes do not encode for TCA cycle genes. Their functions are as yet unknown. These results encouraged us to investigate whether the analysis of other *aci*⁺ mutants will lead to the isolation of new yeast genes.

MATERIALS AND METHODS

Strains and plasmids

The plasmids and *S. cerevisiae* strains used in this study are described in Table 1. Yeast strain D273-10B/A1 *MATa, met*⁻ was the parental strain of the *aci*⁺ mutants. Isogenic strain F87-24b *MAT a his*⁻ was used in standard genetic analysis. *E. coli* strain DH5 α (supE44 Δ lac169 (Φ 80lacZ Δ M15) hsdR17 recA1 endA1 gyrA96thi1 relA1) was used for molecular cloning [19].

Media

The following media were used: YPG – 1% bactopecton, 1% yeast extract, 2% glucose; CK medium [5] – 1% bactopecton, 1% yeast extract, 0,5% glucose, 30 mg/l bromocresol purple (used for mutant selection); and minimal medium G⁰ – 0.67% yeast nitrogen base, 2% glucose. If there was a need, the medium was supplemented with nutritional requirements at a final concentration of 20 μ g/ml. The sporulation medium was – 0.25% yeast extract, 0.1% glucose, 1% potassium acetate. *E. coli* was grown in LB medium [20]. Ampicillin was added to a final concentration of 100 μ g/ml when required. The plating media contained 2% agar (Difco).

Mutagenesis and mutants selection

Mutants were obtained by EMS mutagenesis, which was performed according to the modified method described by Fink [21]. Mutants excreting acid metabolites to the medium were marked as *aci*⁺. The selection was based on the colour change of bromocresol purple, which is violet in a neutral environment, but is yellow in an acid environment. Mutant colonies on the CK medium formed yellow zones.

Genetic analysis

Standard yeast genetic techniques, such as mating, isolation of diploids, sporulation, asci dissection, genetic complementation and tetrad analysis were recommended by Sherman *et al.* [22].

Transformation and gene cloning

Three chosen mutants were subjected to molecular analysis. First, an allele *ura3 Δ 0* was introduced to the *aci*⁺ mutants by crossing with the strain BY4741, and the corresponding spores *aci*⁺ Δ ura were isolated. These were used as the

recipient in transformation with the yeast bank genome on plasmid pFL44L, obtained from the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw. The transformation of yeast cells was performed using the lithium acetate method [23]. Transformants were selected on prototrophy and screened for the *Aci*⁻ phenotype. Yeast total DNA was isolated from the transformants using the method of Kaiser *et al.* [24], and transformed into *E.coli* DH5 α [20]. Next, plasmid DNA was isolated and fragments of yeast genome ends were sequenced by the DNA Sequencing and Olinucleotide Synthesis Laboratory of Polish Academy of Sciences in Warsaw. The yeast genes on the corresponding fragment were identified via the BLAST network service [25].

Tab. 1 Strains and plasmids.

Strains/plasmids	Genotype/description	Source
D273-10B/A1	MAT α met6	Grenson
F87-24b	MAT α his ⁻	Grenson
WY-91	MAT α arg ⁻	Slonimski
BY4741	MAT α his3 Δ 1 leu2 Δ 0 met15 Δ 0 ura3 Δ 0	[26]
EG8	MAT α aci ⁺ met ⁻	this study
EG20	MAT α aci ⁺ met ⁻	this study
EG51	MAT α aci ⁺ met ⁻	this study
pFL44L	Multicopy, shuttle vector, Amp ^R , URA3	[27]
pEG8	5.4 kb insert containing LTV1, MRP8 and SDH3 genes cloned in the BamHI site of pFL44L	this study
pEG20	5.4 kb insert containing LTV1, MRP8 and SDH3 genes cloned in the BamHI site of pFL44L	this study
pEG51/2	3.4 kb insert containing QCR10, LEU5 and YHROO3C genes cloned in the BamHI site of pFL44L	this study
pEG51/3	6.5 kb insert containing QCR10, LEU5, YHROO3C NEM1 and GPA1 genes cloned in the BamHI site of pFL44L	this study

RESULTS AND DISCUSSION

Analyses of *aci*⁺ mutants by classical genetics methods

aci⁺ mutants of *S.cerevisiae* were isolated after ethyl methane sulfonate mutagenesis (2% EMS in 50 mM potassium phosphate buffer, pH 7.0, 90 min, 30°C) of the wild-type strain D273-10B/A1. Those clones which produced a clear yellow zone around colonies grown on the CK medium were selected. The mutants were crossed with the isogenic strain F87-24b, and the diploids selected on prototrophy were checked for their ability to excrete acid metabolites. All the

selected diploids failed to excrete acid metabolites to the CK medium, indicating that the observed Aci^- phenotypes are caused by recessive mutations.

Each of the diploids was sporulated, and the tetrads were analyzed for segregation of acid metabolites excretion, as well as for nutritional markers and mating type. Acids secretion and other characteristics segregated 2:2, which indicates that they resulted from recessive single-gene nuclear mutation.

A test for allelism, performed by crossing each mutant to every other, showed that the analyzed aci^+ mutants can be divided into 16 complementation groups (Table 2). The identification of the 16 complementation groups suggests the existence of at least 16 different genes (cistrons) responsible for the aci^+ feature. Some of the genes were represented by single mutants, others by two, three or four mutants.

Tab. 2. The distribution of the aci^+ monogenic mutants into complementation groups. The procedure of genetic selection used in this study showed that the aci^+ mutants are the results of single-gene nuclear mutations. The mutations are recessive and allelism test grouped them into 16 complementation groups. It suggests that at least 16 different genes are responsible for the aci^+ feature.

Complementation groups	Number of mutants	Representative mutants
I	4	EG7, EG9, EG25, EG33
II	1	EG13
III	2	EG23, EG96
IV	1	EG37
V	3	EG40, EG46, EG92
VI	3	EG47, EG72, EG90
VII	2	EG77, EG86
VIII	1	EG84
IX	2	EG87, EG95
X	2	EG85, EG42
XI	1	EG43
XII	1	EG51
XIII	2	EG24, EG8
XIV	2	EG20, EG6
XV	1	EG30
XVI	1	EG16

Molecular genetic methods

Three aci^+ mutants, EG8, EG20 and EG51, respectively representing the complementation groups XIII, XIV and XII were subjected to further physiological [28] and molecular analysis. In order to isolate fragments of the

yeast genome complementing the aci^+ character of the corresponding mutants, we screened the yeast genomic library on the multicopy plasmid pFL44L among ura^+ transformants. The uracil prototrophic transformants were tested on the CK medium for Aci^- character. In total, four colonies without a yellow zone on violet CK medium (Aci^- phenotype) were found and isolated: one transformant of EG8 and one of EG20 (tEG8, tEG20), and two transformants of EG51 (tEG51/2, tEG51/3). Plasmid DNA was prepared from each of the four independently isolated yeast clones and after amplification in *E.coli*, the plasmid DNA was used in retransformation of the respective mutant yeast strains. Aci^- phenotype was observed in 100% of the colonies of the transformants. Restriction analysis performed on plasmid DNA from each of four clones revealed that the patterns produced from two clones, designated pEG8 and pEG20, were identical but different from the pEG51/2 and pEG51/3 clones. The DNA of pEG51/2 and pEG51/3 plasmids were of different lengths.

The nucleotide sequence of the 5' and 3' ends of the pEG8, pEG20, pEG51/2 and pEG51/3 inserts were determined. The fragments complementing Aci^+ phenotype of the EG8 and EG20 mutants were similar. These fragments appeared to be a part of the XI chromosome containing three genes *LTV1*, *MRP8* and *SDH3* (Fig. 1). Mutation in *SDH3*, the gene coding one of the subunits of succinate dehydrogenase, is probably responsible for the aci^+ character of the mutants. Plasmids pEG51/2 and pEG51/3 appeared to contain overlapping fragments of the VIII chromosome (Fig. 2). The overlapping fragments contain two ORFs, named *LEU5* and *YHR003C*, of unknown function; they are good candidates to possess aci^+ mutation. Further identification research is in progress. The functional analysis and disruption of the sixteen genes responsible for the Aci^- phenotype is a time-consuming program. We started from four genes selected at random and the preliminary results obtained with two of them are presented in this study. In the case of mutants EG8 and EG20, *SDH3* is the gene which gives the Aci^+ phenotype when mutated, although this supposition required further confirmation. The results obtained with mutant EG51 are more interesting, because the mutations in two genes of unknown function (*LEU5* and *YHR003C*) probably lead to medium acidification.

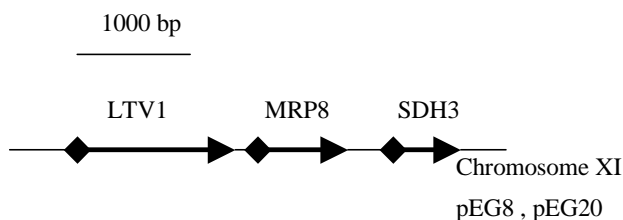


Fig. 1. The inserts isolated from plasmids pEG8 and pEG20. The inserts isolated from plasmids pEG8 and pEG20 were identical.

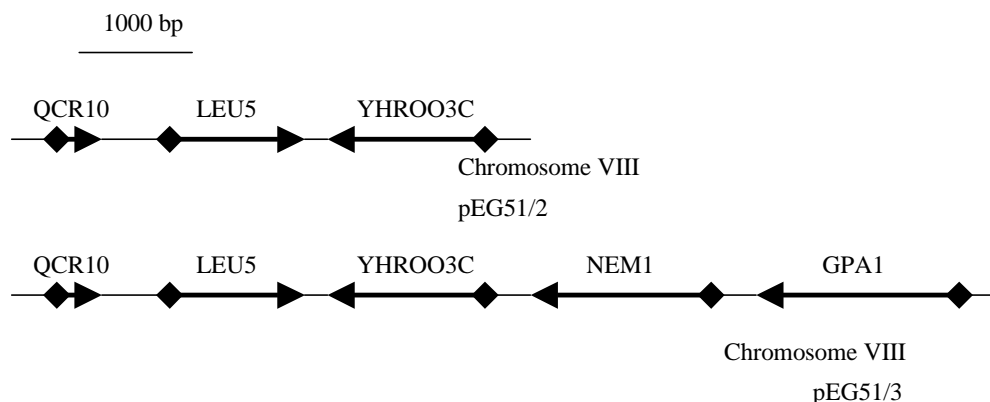


Fig. 2. The inserts isolated from plasmids pEG51/2 and pEG51/3. The inserts isolated from plasmids pEG51/2 and pEG51/3 were different but overlapping.

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