GENE CONTROL OF PIGMENTATION ASSOCIATED WITH A SPECIFIC LYSINE REQUIREMENT OF SACCHAROMYCES[1]

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Sixty-eight different lysine-requiring mutants of Saccharomyces (which had been produced by ultraviolet irradiation, nitrous acid, 5 bromo-uracil and acridine yellow) were located at twelve different loci by complementation tests. A mutant at one of the loci (ly_9) produced a bright yellow pigment, a phenotype not commonly encountered in yeast mutants. Genetical and biochemical studies of pigment-production associated with the ly_9 auxotroph seemed to merit further study.

Experimental Methods

Haploid strains of opposite mating types with desired markers were used for preparing hybrids. Genetic analysis of tetrads was made by dissecting 4-spored asci from the respective hybrids with a micromanipulator. The segregants were tested in complete synthetic media lacking only the specific nutrilite for which they were being tested. Segregation of the yellow pigment was followed better on glucose nutrient agar plates, hence, the progeny was also replicated on glucose nutrient agar plates which served both as test plates for segregation of pigment and control plates for all segregants.

Pigment was isolated from a 72-hour old culture (B3003) having the phenotype lysine-requiring and yellow-colored, and was grown on glucose nutrient broth through shaking. Cells were harvested by centrifugation and treated with various solvents for one hour with constant stirring. The relative

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extractability of different solvents was ascertained by colorimetric reading. Whatman No. 1 paper and a glass jar were used for paper chromatography.

Results

The dominant phenotype: The lysine-requiring yellow strain (B3003, ly_9) was plated on glucose nutrient agar for single cell isolation and subsequently replicated on (1) complete synthetic agar without lysine and (2) on glucose nutrient agar (control). After 48 hours incubation at 30° C, a mixture of white and yellow colonies (87:70) appeared on the control plate, while no colonies appeared on the lysine-deficient synthetic agar, indicating that the white colonies were not revertants of the lysine auxotroph, but rather back mutations to the wild type phenotype of pigmentation due to an event having no impairment on lysine. Single cell colonies of both white and yellow strains were purified by spreading and respreading to the point where vegetative stability of either trait was confirmed.

Genetic and biochemical behavior of the pigment: A hybrid (B2923α, ur, yel, ly x B3003-1 a, ad, YEL, ly) was made between the white and a yellow strain, both of which were lysine-requiring. Colonies from single diploid cells isolated by the micromanipulator were white in color, indicating the dominance of white over yellow. Analysis of 28 tetrads from this hybrid revealed a 2:2 segregation of yellow and white (Table 1A), thus demonstrating that the pigment phenotype (yellow) is gene controlled. All the segregants required lysine. Mendelian segregation was also exhibited by uracil, adenine and mating-type loci. However, yellow segregants having simultaneous requirements for adenine and lysine were a deeper yellow than the adenine-independent ones (Table 1B). Simultaneous requirements for uracil and lysine had no effect on yellow-color segregants.

To determine if the pigment is dependent or independent of ly_9, a hybrid was made between ly_4 (B6482 a, ly_4, ur) and a yellow culture (B6043α, ad, ly_9). The result is given in Table 2. Only two segregants of each parental ditype tetrad (+ly_4, +ly_4, ly_9+, ly_9+) were yellow; one segregant of each tetratype tetrad (ly_9, ly_4, ++, +ly_4, ly_9+) was yellow; none of the segregants of the

Table 1. A - Segregation of yellow (y) pigment. B - Interaction between adenine requirement and yellow segregants (y^D = deeper yellow).

Hybrid	No. of asci	Segregants a	b	c	d	Color
A						
B2923 yellow (α, y, ly_9 AD, ur)	24	ly(y)	ly(y)	ly(Y)	ly(Y)	2 yellow:2 white
X	2	ly(y)	ly(y)	ly(y)	ly(Y)	3 yellow:1 white
B3003-1 white (a, Y, ly_9 ad, UR)	1	ly(y)	ly(Y)	ly(Y)	ly(Y)	1 yellow:3 white
B						
Interaction between yellow and adenine requiring mutant	2	ly ad(y^D)	ly ad(y^D)	ly AD(Y)	ly AD(Y)	2 yellow:2 white
	15	ly ad(y^D)	ly AD(y)	ly ad(Y)	ly AD(Y)	2 yellow:2 white
	6	ly AD(y)	ly AD(y)	ly ad(Y)	ly ad(Y)	2 yellow:2 white
	1	ly ad(y^D)	ly ad(Y)	ly ad(Y)	ly AD(y)	2 yellow:2 white

nonparental ditype tetrad (ly_9, ly_4, ly_9, ly_4, ++, ++) were yellow. The yellow segregants were found to be ly_9 by the complementation test.

The pigment is not excreted in the growth media, in contrast to the colored complex described by Mattoon et al. (1962). The color disappears on treatment with weak acid, but alkali has no apparent effect on it. This pigment can be extracted by hot water, ethanol, acetone and chloroform. The chromatograms of the colored fraction showed more than one ninhydrin-positive, fluorescent spot with both phenol water and butanol acetic acid solvents.

Discussion

Few cases of irregular segregation were observed. Two tetrads showed an excess of yellow (yellow:white = 3:1), one tetrad showed an excess of adenine deficiency (ad:AD = 3:1) and one an excess of white (yellow:white = 1:3).

These irregular segregations are due to gene conversion (Lindegren, 1953) in the respective loci. Pigmented segregants requiring adenine were a deeper yellow due to the interaction between the adenine precursor and the pigment. A comparable interaction between the pink color of ad_1 and methionine requirement has been observed previously (Lindegren, 1949). Both mutation and conversion at the locus controlling pigment production are independent of the locus controlling the synthesis of lysine, indicating that pigmentation and lysine requirements are not one trait controlled by a single gene. On the contrary, the original yellow mutant is a double mutant for lysine requirement and pigment production, each of which is controlled by a single gene, although the expression of pigment production is only possible in the presence of lysine requirement.

Data from Table 2, together with the complementation test, show that the pigment production is specifically associated with ly_9 and not with ly_4.

Table 2. Dependence of yellow pigment on lysine requirement controlled by a particular locus (ly_9).

Hybrid	No. of asci	Segregants a	b	c	d	Color
B6482 (a, ly_4 AD, ur)	2	ly	ly	ly	ly	2 yellow:2 white
X	12	ly	LY	ly	ly	1 yellow:3 white
B6043 yellow (α, ly_9, ad, UR, y)	7	LY	LY	ly	ly	0 yellow:4 white

Saunders et al. (1964) have shown that ly_9 lacks the enzyme following α-aminoadipic-δ-semialdehyde, an unstable intermediate along the α-aminoadipic acid pathway (Mitchell and Houlahan, 1948) leading to lysine. Hence the pigment is postulated to be a gene-controlled derivative of α-aminoadipic-δ-semialdehyde in its stabilized form (Figure 1). However, its confirmation awaits purification and characterization of the pigment.

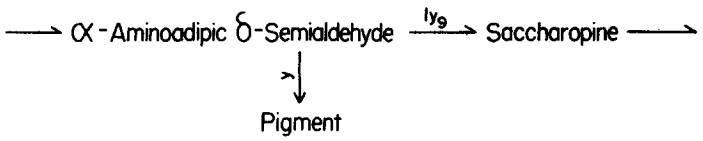

FIG.1 Step leading to the formation of pigment from lysine(ly_9) precursor.

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