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Abstract

Haploid yeast cells were exposed to ultraviolet and the survivors tested for nutritional requirements. A total of more than 5000 colonies was tested against twenty-three nutritional requirements, and a group of 138 mutants was obtained. These mutants have all been characterized with respect to their nutritional requirement, and many of them have been identified with particular genetic loci. Biosynthetic studies have been performed on some of the mutants. Three of the mutants were shown to be centromere-linked, and these were further shown to be linked with certain other known centromere-linked genes.

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Introduction

The sporogenous yeast Saccharomyces cerevisiae has been used in genetic and radiation studies for a number of years. Relatively stable haploid, diploid, and polyploid cultures are available which make this organism particularly suited to studies of diploid and polyploid meiosis by tetrad analysis,[2, 7, 11] spontaneous and induced mitotic crossing-over,[4, 5, 12] and gene conversion.[8] Although nutritional markers already are available in various yeast stocks, it was felt that their numbers were small compared with expectations based on the assumption of similar biosynthetic pathways in yeast and, for instance, in Neurospora, for which large numbers of biochemical blocks are available. A larger selection of biochemical mutants will aid in a comparison of biosynthetic pathways in yeast and other microorganisms, and in addition will provide genetic markers that can eventually be incorporated into the linkage maps of Saccharomyces.[2, 7] This report describes methods and results of a search for biochemical mutant strains, as well as some preliminary biosynthetic and genetic studies with some of the strains.

Material and Methods

A single haploid strain of Saccharomyces cerevisiae was used in all the mutation experiments. Its designation is S 288 C and it was derived from a spore isolate of a cross between strain 93-1Cα (Lindegren) and strain 1198-1b(Hawthorne). This culture has a normal cytochrome complement and is nonclumpy (i.e., a 48-hour slant growth suspends with slight shaking into a population of cells containing more than 98 percent single or budding cells). The culture has no amino acid, purine, or pyrimidine requirements, and grows satisfactorily on a synthetic medium composed of salts, trace elements, vitamins, and carbon source. It could easily be identified by its mating type (α) and by its inability to ferment maltose and galactose (due to the presence of the g-2 block[3]). Irradiation was performed with a low-pressure mercury vapor lamp at an intensity of 36 ergs/mm^2/sec. Cells were plated onto the surface of complete medium (YEPD: 1 percent yeast extract, 1 per cent peptone, 2 per cent dextrose, 2 per cent agar) contained in a 10 cm Petri dish, irradiated, and then incubated at 30° C for 3 to 4 days. At this time, colony counts were made and the percentage of viable cells determined. These colonies, representing cells that had survived the exposure, were then tested for nutritional requirements.

The method of testing for mutants was as follows: colonies were sampled near the edge with a small loop and streaked onto two synthetic test media contained in Petri dishes. The two media used to identify mutants were (a) minimal medium: composition identical to Difco Yeast Nitrogen Base less methionine, tryptophane, and histidine; (b) synthetic complete medium: minimal medium enriched with 18 amino acids, adenine, uracil, thymine, guanine, and cytosine; the supplements were added to produce a final concentration of 20 mg/liter (later studies revealed that threonine was required at a concentration of 500 mg/liter). Mutants were identified by their inability to grow on minimal medium in contrast to their ability to grow on synthetic complete medium. Some colonies failed to grow on either synthetic medium but were able to grow on YEPD. It is to be noted that vitamins were included in both synthetic media and thus vitamin auxotrophs were not screened. The above method is analogous to the total isolation procedure of Pontecorvo.[9] The replica plating method of Lederberg and Lederberg[6] was tried in some initial experiments with an apparently lower yield of mutants, perhaps owing to sectoring of the mutant colonies. The delayed-enrichment technique was tried, with unsatisfactory results.

A number of pink colonies, all possessing blocks in adenine synthesis,[10,12] were isolated directly, but their frequencies cannot be compared to those of other loci, since essentially they were selected from a much larger sample.

The nutritional requirements of the mutants were determined by a second screening on a series of plates containing minimal medium supplemented with different groups of 5 to 10 of the nutrients, followed by a final screening with single supplements. Later in the study, as it was seen that certain requirements were evidenced more frequently than others, it was found more efficient to screen on a series of plates containing minimal medium supplemented with all but one of these nutrients, with a different nutrient omitted from each plate in the series.

In order to characterize the genetic locus associated with the nutritional requirement, it was necessary to perform allelism tests. A number of the mutant cultures were incorporated into crosses, and segregants were isolated that were of opposite mating type but had the same nutritional requirements. With these segregants, and also with a number of tester stocks provided by Drs. Roman and Hawthorne, allelism tests were performed. Mutants possessing a particular requirement were mated in all combinations with testers of the opposite mating type but same requirement. Allelism between two strains was indicated by the inability of the diploid cross to grow on media lacking the requirement common to both parents. Conversely, two loci were considered nonallelic when the cross was able to grow.

Results

The numbers and frequencies of mutants isolated at different doses of ultraviolet are summarized in Table I. Only those mutants which were stable after three successive transfers are included in the results. About one-third of the presumptive mutants isolated on the first screening failed to show a requirement on the second or third screening. The numbers of pink colonies isolated also are omitted from the results.

Table I

Frequency of nutritional mutants in Saccharomyces cerevisiae as a function of ultraviolet dosage.

Dose (sec)	Percent survivors	Colonies tested	No. of mutants	Mutants per survivor (percent)
0	100	130	0	0
10	62	183	4	2.2
20	10	637	11	1.7
30	2	1259	23	1.8
40	0.4	1453	40	2.7
50	0.1	1248	40	3.2
60	0.02	311	12	3.8
70	0.002	116	0	0
80	0.002	36	0	0
Cumulative		5473	129	2.36

The frequency of mutants is seen to increase with exposure up to at least 60 seconds, which corresponds to a viability of 2 in 10^4. Failure to obtain mutants at higher doses could be indicative of a decrease in mutation frequency, as is observed in many similar studies, but may be due simply to the small sample size. In the lower exposure region, the data, though suggestive, are not adequate to establish a linear relation between mutation frequency and dose. It is interesting to compare preliminary results on mutation to canavanine resistance[13] with the results shown in Table I for the same culture under similar conditions. In contrast to the above results, the frequency of canavanine-resistant mutants reaches a maximum ($\sim 2/10^6$ survivors) at an exposure of only 15 seconds and falls off rapidly at higher doses. No mutants could be detected ($< 1/10^7$ survivors) at doses greater than 30 sec.

The distribution of the mutants with respect to nutritional requirements and genetic locus (allele) is summarized in Table II. Some of the cultures classified under a single nutritional requirement have one or more additional requirements. These will be described in the section on biosynthetic studies. Cultures allelic with loci already characterized were given their previously assigned designation. Mutants not allelic with known markers were given new designations. For instance histidine-1, histidine-2, and histidine-3 correspond to alleles present in cultures obtained from Drs. Roman and Hawthorne. Histidine-4 through histidine-8 correspond to new alleles found in the group of 22 histidine mutants. Other alleles made available for testing were: adenine-1 to adenine-7, methionine-1, tryptophane-1 and tryptophane-2, and uracil-1.

Table II

Distribution of ultraviolet-induced mutants of Saccharomyces cerevisiae with respect to nutritional requirements and genetic locus.

Requirement	Number of mutants	Genetic locus (allele)								
		1	2	3	4	5	6	7	8	unidentified
Adenine	32	4	16	2	1	1	5	3	-	-
Arginine	9	2	1	-	1	1	3	-	-	1
Cysteine	1	1	-	-	-	-	-	-	-	-
Glutamic	2	1	-	-	-	-	-	-	-	1
Histidine	22	1	1	2	4	4	5	2	1	2
Isoleucine	3	3	-	-	-	-	-	-	-	-
Leucine	4	2	1	-	-	-	-	-	-	1
Lysine	14	3	4	1	3	-	-	-	-	3
Methionine	23	-	3	1	-	-	-	-	-	19
Threonine	5	1	3	1	-	-	-	-	-	-
Tryptophane	10	-	2	1	1	-	-	-	-	6
Tyrosine	4	1	2	1	-	-	-	-	-	-
Uracil	9	1	3	-	-	-	-	-	-	5
YEPD	11									
Total	149									

Biochemical Studies

Some preliminary studies on biosynthetic pathways in yeast have been undertaken using this group of mutants. Of 11 methionine mutants tested, all but two grew on homocystine. These two, which are nonallelic, could be supplemented only with methionine. Some of the tryptophane mutants could be supplemented with anthranillic acid, others with indole, and one with just tryptophane. The mutants corresponding to the threonine-2 allele have a double requirement of threonine and methionine. These mutants are similar to ones found in Neurospora. Of the tyrosine mutants, tyrosine-1 corresponds to a single requirement of tyrosine, tyrosine-2 corresponds to a requirement of both tyrosine and phenylalanine, and tyrosine-3 corresponds to a multiple requirement of tyrosine, phenylalanine, tryptophane, and para-amino-benzoic acid. The block identified as adenine-3 (and also histidine-3) confers a double requirement of adenine and histidine.[12] All the above blocks conferring multiple requirements have been shown to segregate as single genetic loci.

These examples are all consistent with findings for corresponding biosynthetic pathways in other microorganisms.

Genetic Analysis

Some of the mutants representing new genetic loci were incorporated into crosses along with other known nutritional and fermentation markers. Asci were dissected and the spores were tested for segregation of the characters introduced into the cross. Standard methods of tetrad analysis were applied to determine centromere and gene-gene linkage. From a cross A B x a b, three types of asci can be obtained: Parental Ditype - PD (A B, A B, a b, a b); Nonparental Ditype - NPD (A b, A b, a B, a B); and Tetratype - T (A B, A b, a B, a b). For two loci A and B segregating independently of each other and of their respective centromeres, the relative frequencies of these types of asci are expected to be PD:NPD:T = 1:1:4. If both A and B are linked to their respective centromeres, the frequency of tetratype asci decreases relative to the other two, i.e., PD:NPD:T = 1:1:< 4. If A and B are linked to each other, the frequency of both NPD and T asci decreases, the former much more rapidly. Thus for gene-gene linkage, PD:NPD:T = 1:<< 1:< 4. The locus tryptophane-1 (tr-1) has been studied previously[1] and has been shown to be closely linked to its centromere. This locus then served as a suitable marker to detect centromere linkage in the unordered asci studied.

Three of an initial group of eleven loci studied showed highly significant centromere linkage, i.e., histidine-4 (his-4), histidine-6 (his-6), and leucine-1 (leu-1). Three others, i.e., lysine-1 (lys-1), threonine-1 (thr-1), and uracil-2 (ur-2), gave data suggestive of linkage. The remainder showed no evidence for centromere linkage. These results are summarized in Table III.

Table III

Segregation of some of the mutants relative to the centromere-linked locus tr-1 in Saccharomyces cerevisiae.

Cross	Distribution of ascus types			
	PD	NPD	T	percent T
his-4 – tr-1	20	17	18	33
his-6 – tr-1	23	28	13	20
leu-1 – tr-1	113	113	11	4.5
lys-1 – tr-1	4	11	21	58
thr-1 – tr-1	9	6	17	53
ur-2 – tr-1	9	8	27	61
arg-1 – tr-1	7	6	25	66
his-5 – tr-1	7	2	20	69
thr-2 – tr-1	3	3	12	67
is-1 – tr-1	2	2	14	78
tr-4 – tr-1	5	9	29	68

The markers showing centromere linkage were incorporated into crosses containing other known centromere-linked genes[1, 2, 7, 12] in order to test for gene-gene linkage. Three cases of linkage were found and the data supporting these linkages are summarized in Table IV.

Table IV

Evidence for gene-gene linkage of centromere-linked genes in Saccharomyces cerevisiae.

Cross	Distribution of ascus types			
	PD	NPD	T	percent T
his-4 – mating type(α)	27	0	35	58
his-6 – lys-1	11	0	19	63
leu-1 – ad-6	25	0	27	52

The above data indicate that his-4 and mating type are on opposite sides of their centromere, as also are lys-1 and his-6. Leu-1 is very close to its centromere.

Many more crosses are being studied, in the hope of obtaining one or more linkage groups containing a number of genetic markers.

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