MUTATORS IN *SACCHAROMYCES CEREVISIAE*: *MUT1-1*, *MUT1-2* AND *MUT2-1*

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ABSTRACT

The purpose of this study was to characterize two mutator stocks of yeast which were induced and selected on the basis of high spontaneous reversion rates of the suppressible "ochre" nonsense allele *lys1-1*. In the mutator stock VA-3, a single mutation, designated *mut1-1*, is responsible for the increase in the reversion rate of the ochre alleles *lys1-1* and *arg4-17*. In stock VA-105, there are two separate mutator mutations. Tetrad analysis data showed these two loci are loosely linked. Based on complementation data, one of these mutations is at the same locus as *mut1-1* and designated *mut1-2*. The second mutator of stock VA-105 was designated *mut2-1*. All three mutators are recessive. Both *mut1-1* and *mut1-2* give a high mutation rate for ochre nonsense suppressor (*SUP*) loci, but not for the ochre nonsense alleles. On the contrary, the mutation rates of the ochre alleles are greatly reduced. With the mutant *mut2-1* there were mutations at both the *lys1-1* site and its suppressors; *mut2-1* is as effective as *mut1-2* but not as effective as *mut1-1* in inducing reversions of a missense mutant, *his1-7*. Neither *mut1-1*, *mut1-2* nor *mut2-1* were effective in inducing reversions of a putative frameshift mutation, *hom3-10*, or in inducing forward mutations to canavanine resistance.

CERTAIN genes in many different organisms have been shown to modify the mutation rates of many loci (for a review, see GENETICS Supplement, April 1973). Although it is now obvious that no one mechanism can account for all types of mutator effects observed, the investigations of a few of these have led to hypotheses concerning specific mechanisms involved in the enhancement of the spontaneous mutation rate.

It has been suggested that enhanced spontaneous mutation rates can be caused by accumulation of abnormal purines or pyrimidines which then induce base analog mutagenesis (KIRCHNER and RUDDEN 1966). Errors in DNA replication due to mistakes made by the natural polymerases have also been implicated. For example, a mutagenic DNA polymerase in bacteriophage T4 (SPEYER 1965; SPEYER, KARAM and LENNEY 1966) causes specific errors in DNA synthesis yielding transition-type base substitutions. HALL and LEHMAN (1968) have described the *in vitro* action of a DNA polymerase mutant of T4 which yielded enhance-

ment of the frequency of transversions. The mutator activity in this locus can be ascribed to reduction or loss of 3′ exonuclease activity (HERSHFIELD and NOSSAL 1973; SCHNAAR, MUZYCZKA and BESSMAN 1973). Normal errors taking place during DNA synthesis are corrected less often if the 3′ exonuclease is defective. Other polymerase mutants result in an antimutator activity (DRAKE *et al.* 1969; DRAKE and ALLEN 1968). Antimutator activity stems from a decrease in the rate of DNA synthesis which permits overcorrection by the 3′ exonuclease.

Great specificity has been attributed to some mutator strains. For example, the Treffers' mutator in *E. coli* K-12 (TREFFERS, SPINELLI and BELSER 1954) causes a unidirectional change—an AT → CG transversion (YANOFSKY, COX and HORN 1966). More recently, COX, DEGNEN and SCHEPPE (1972) have demonstrated that another *E. coli* mutator gene, *mutS1*, yields transitions, confirming the mutagen specificity work of SIEGEL and BRYSON (1964). However, they showed that not all A:T pairs are equally susceptible; thus, nearest neighbors may influence the mutator action.

The implication of misrepair in the internal control of mutation rate by the cell has also been related to the discovery that repair-deficient strains of fungi exhibit mutator activity (*Saccharomyces cerevisiae*: ZAKHAROV, KOZINA and FEDEROVA 1968; VON BORSTEL *et al.* 1968: *Schizosaccharomyces pombe*: LOPRIENO 1972; and *Neurospora crassa*: DE SERRES 1971). Furthermore, in the yeast *S. cerevisiae*, MAGNI and VON BORSTEL (1962) found an increase in the spontaneous mutation rate at meiosis (the "meiotic effect"). Particular mutations exhibited a marked increase in reversion correlated with outside marker exchange (MAGNI 1963). MAGNI suggested that spontaneous mutations during meiosis are caused by misalignment of bases during the recombination process resulting in addition-deletion mutations. In support of this hypothesis, no meiotic effect is observed with reversion of known base substitutions (VON BORSTEL, BOND and STEINBERG 1964; MAGNI 1964, 1969).

In order to study further the problem of genetic control of mutation rates in yeast, VON BORSTEL *et al.* (1973) formulated a program to select mutants solely on the basis of enhanced mutation rates, without initial reference to selection on the criteria of altered DNA replication, radiation sensitivity, or any other pleiotropic effect. Preliminary reports of these studies have appeared which partially characterize some of the 111 presumptive mutator mutants (VON BORSTEL, *et al.* 1971; GOTTLIEB 1972; VON BORSTEL *et al.* 1973).

The present report deals with the analysis of two stocks, VA-3 and VA-105, which had among the highest mutation rates of the many tested. It will be shown that VA-3 has one mutator gene present (*mut1-1*), while VA-105 has two mutator genes (*mut1-2* and *mut2-1*). In addition, the specificity of the mutator effect for reversion of suppressible nonsense alleles of the ochre variety will be shown, as well as the level of effect on reversion of presumptive missense and frameshift mutants, and finally, on forward mutation to canavanine resistance.

MATERIALS AND METHODS

Strains: The two mutator strains used in these studies (VA-3 and VA-105) are two of the strains briefly described by VON BORSTEL *et al.* (1973). These mutator strains were induced by

ethyl methanesulfonate treatment of strain X1687-12B obtained from R. K. MORTIMER. The five markers in this strain, *lys1-1*, *arg4-17*, *trp5-48*, *ade2-1* and *his5-2* are all ochre nonsense mutants (GILMORE, STEWART and SHERMAN 1968; HAWTHORNE 1969). The other alleles used in these studies are *his1-7* (believed to be a missense mutation), *hom3-10* (a presumptive frameshift mutation) and canavanine resistance (probably mutations of arginine permease). The genotypes of the haploid and diploid strains used in this study are listed in Tables 1 and 2. (For a description of the gene symbols see PLISCHKE *et al.* 1976.)

It is important to note that all strains used are psi$^-$ (COX 1965). The original strains were from the laboratories of R. K. MORTIMER and G. E. MAGNI, both of whom did not have psi$^+$ in their stocks when the original strains were acquired. Furthermore, *trp5-48*, which is suppressed in the presence of psi$^+$, was maintained in the strains being tested for mutator activity.

Media: Most of the media used in these studies have been described previously (VON BORSTEL, CAIN and STEINBERG 1971; VON BORSTEL *et al.* 1973). The canavanine medium (CAN) is synthetic complete (MC) minus arginine supplemented with canavanine sulfate at a concentration of 60 mg/1.

Detection of mutator mutants in crosses: A simple test for rapid detection of mutator strains is routinely used. The test has been described previously (VON BORSTEL *et al.* 1973). Briefly, a suspension of between 10^6 and 10^7 cells/ml of a lysine-requiring strain is spread on one plate each

TABLE 1

*Genotype of haploid strains**

Strain	Genotype								
X1687–12B	α		*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*		
X1687–16C	a		*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*	*leu1–12*	*met1*
VA-3	α	*mut1–1*	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*		
VA-105	α	*mut1–2, 2–1*	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*		
XV151–6D	a				*his1–7*				
XV163–3C	α		*ade2–1*	*arg4–17*		*lys1–1*	*trp5–48*		*hom3–10*
XV169–1A	α		*ade2–1*		*his1–7*	*lys1–1*	*trp5–48*		*hom3–10*
XV169–15A	a		*ade2–1*	*arg4–17*	*his1–7*	*lys1–1*	*trp5–48*		*hom3–10*
XV173–8B	α	*mut1–1*	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*		
XV173–14D	a	*mut1–1*	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*	*leu1–12*	*met1*
XV173–17C	α	*mut1–1*	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*	*leu1–12*	
XV173–24B	a	*mut1–1*	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*		*met1*
XV174–1C	a	*mut1–2, 2–1*	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*	*leu1–12*	
XV177–23A	α	*mut1–1*	*ade2–1*	*arg4–17*	*his1–7*	*lys1–1*	*trp5–48*		*hom3–10*
XV177–23D	a	*mut1–1*	*ade2–1*	*arg4–17*	*his1–7*	*lys1–1*	*trp5–48*		*hom3–10*
XV181–3A	a	*mut2–1*	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*		
XV181–3C	α	*mut1–2*	*ade2–1*	*arg4–17*	*his1–7*	*lys1–1*	*trp5–48*		*hom3–10*
XV181–3D	a	*mut1–2*	*ade2–1*	*arg4–17*	*his1–7, 5–2*	*lys1–1*	*trp5–48*		*hom3–10*
XV185–1D	–		*ade2–1*	*arg4–17*	*his1–7*	*lys1–1*	*trp5–48*		*hom3–10*
XV185–3C	–		*ade2–1*	*arg4–17*	*his1–7*	*lys1–1*	*trp5–48*		*hom3–10*
XV185–6A	–		*ade2–1*	*arg4–17*		*lys1–1*	*trp5–48*		*hom3–10*
DG15–7D	a		*ade2–1*	*arg4–17*	*his1–7*	*lys1–1*	*trp5–48*		*hom3–10*
DG16–1A	a	*mut2–1*	*ade2–1*	*arg4–17*		*lys1–1*	*trp5–48*		
DG16–1B	a	*mut1–2*	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*		*hom3–10*
DG16–1D	α	*mut1–2*	*ade2–1*	*arg4–17*	*his1–7*	*lys1–1*	*trp5–48*		
DG16–2D	α	*mut1–2, 2–1*	*ade2–1*	*arg4–17*	*his*(?)	*lys1–1*	*trp5–48*		*hom3–10*
DG31–1B	–	*mut1–1*	*ade2–1*		*his*(?)	*lys1–1*	*trp5–48*		*hom3–10*
DG50–4C	–	*mut2–1*	*ade2–1*			*lys1–1*	*trp5–48*		
DG50–8B	–	*mut2–1*	*ade2–1*	*arg4–17*	*his1–7*	*lys1–1*	*trp5–48*		*hom3–10*

* Strains X1687–12B and X1687–16C are from R. K. MORTIMER.

TABLE 2

Origin of diploid strains

Strain	Origin	Strain	Origin
XV10	X1687–16C	DG15	XV169–15A
	X1687–12B		X1687–12B
XV169	XV151–6D	DG16	XV169–15A
	XV163–3C		VA-105
XV173	X1687–16C	DG17	XV173–14D
	VA-3		VA-105
XV174	X1687–16C	DG21	XV181–3A
	VA-105		XV173–17C
XV177	XV169–15A	DG31	XV173–24B
	VA-3		XV169–1A
XV181	XV169–15A	DG44	DG16–1B
	VA-105		DG16–1D
XV185	XV169–15A	DG46	DG16–1A
	X1687–12B		DG16–1C
DG7	XV173–14D	DG50	DG16–1A
	VA-3		XV169–1A
DG8	XV174–1C	DG52	X1687–16C
	VA-3		X1687–12B
DG10	XV174–1C	DG56	DG16–1B
	VA-105		XV173–8B

of MC and lysine omission medium. At 20 μg/ml of lysine in MC, lysine limits the growth of the strain. In a few days the plate is covered by a uniform, but thin, "lawn" of yeast. Revertants to lysine independence continue to grow. Strains containing mutator mutants exhibit a much higher frequency of colonies on MC plates than do control strains.

Another simple test that has been used for preliminary identification of mutator genes was adapted from the method originally developed for detecting mutator mutants (VON BORSTEL *et al.* 1973). The spore products from dissected asci are streaked onto complete medium, grown, and replica-plated on complete medium as well as omission media for marker determination. The next day the replica on complete medium is replica-plated on another lysine omission plate. After this double replication, the mutator strains have accumulated more revertants to lysine independence than have nonmutator strains, and these revertants show as multiple growing spots on the streak on the lysine omission plate. Two or more revertant spots in a short streak can usually be taken as evidence that the mutator gene is present.

Revertant analysis: In some of the experiments involving ochre-suppressible mutants, revertant colonies were further analyzed by picking the colonies, allowing them to grow on the appropriate omission medium, and then replica-plating to complete, minimal and omission media for each of the requirements of the particular strain. If the revertant grows only on the lysine omission plate, it is considered to be a reversion at the *lys1-1* site itself.

A second method used to distinguish between *SUP* mutations and mutations at the original ochre allele has been described in detail by SCHULLER and VON BORSTEL (1974). When the adenine concentration is lowered from 20 mg/l to 5 mg/l, the adenine becomes depleted and those revertants which still require adenine will accumulate a red pigment. The lysine becomes limiting long before the adenine, thus there is no selection for adenine reversion. They showed that the vast majority of the red colonies are locus revertants. This method has the advantages that mixing or sectoring of locus mutants with ochre suppressor mutants will not affect the results, and the problem of unconscious selection in favor of suppressor mutations is overcome.

Measurement of revertant frequency and computation of mutation rates: (1) P_0 method: The method for measuring the revertant frequency in compartmented culture boxes has been described by VON BORSTEL, CAIN and STEINBERG (1971). This 1000-compartment fluctuation test

in which growth is restricted by limiting the amount of the relevant requirement was used for reversion rate determination of *lys1-1*, *his1-7* and *arg4-17*. Revertants can continue to grow and form visible colonies after the limiting substance has been depleted. At the end of 12 days incubation at 26° the compartments containing colonies are recorded.

It is assumed that the number of revertant colonies in the independent compartments follows a Poisson distribution. Since there could be difficulties determining the other terms accurately, the P_0 term (the fraction of compartments which contain *no* revertant colonies) is used to calculate the reversion rates in this method. This method for determining mutation rates is based on the analysis of mutation by LURIA and DELBRÜCK (1943). Values for the mutation rates were obtained using an appropriate computer program.

(2) Method of median: The method described above was not suitable for determining reversion rates of *hom3-10* and forward mutation rates to canavanine resistance. A fluctuation test utilizing 15 independent cultures was used for this study. The method is a slight modification of that described by MAGNI and VON BORSTEL (1962).

The mutation frequency per cell per division (mutation rate) was calculated by the method of the median of LEA and COULSON (1949). For the estimation of the mutation rates in these experiments, residual growth was assumed not to take place; this leads to a slight overestimation of the spontaneous rates in all strains.

RESULTS

Tetrad analysis: The two mutator stocks, VA-3 and VA-105, were crossed to wild type and the hybrids sporulated. Asci were dissected to analyze segregation and recombination in tetrads. One mutator gene segregated in mutator stock VA-3 (VON BORSTEL *et al.* 1973). This mutator was designated *mut1–1*.

Stock VA-105 contains two mutator mutations which are loosely linked, because the nonparental ditypes are significantly less frequent than the parental ditypes [$p(x^2) \simeq .0005$] (PD = 13, NPD = 2, and T = 19). There is no evidence for centromere linkage for either of the mutant alleles. When these two mutator mutations were tested by complementation with *mut1–1*, it was found that one of them was at this locus (see Table 3). This mutator mutant was designated *mut1–2*, and the other mutator gene was designated *mut2–1*.

Reversions of ochre-suppressible mutants: Reversions of the ochre nonsense mutants *lys1–1* and *arg4–17* can be back mutations at the mutant site itself or forward mutations at suppressor loci (*SUP*), thought to encode tRNA genes. Reversion data for these two ochre mutants are shown in Tables 4 and 5. It is clear that both suppressible ochre sites respond similarly to the action of the mutators. Since the data are more extensive for reversions of *lys1–1*, these will be considered in detail.

It is of interest to note that *mut1–1* and *mut1–2* have high mutation rates for the *SUP* loci, but not for the ochre nonsense allele. Quite the converse, the mutation rate at the site of the ochre allele *lys1–1* is greatly reduced; *mut1–1* and *mut1–2* probably act as antimutators at the locus itself.

Although the data are limited, *mut2–1* appears to differ from *mut1–1* and *mut1–2* in its action. In *mut2–1* strains high mutation rates at the *SUP* loci are also observed, although not as high as in *mut1* strains. The mutants, *mut1–2* and *mut2–1* in combination produce an effect on the *SUP* loci which is at least additive.

TABLE 3

Total spontaneous mutation rates of the ochre mutant lys1-1 *and its suppressors in diploid strains*

Strain	Genotype	Mutation rate ($\times 10^8$)	Total number of revertant compartments
XV10	*MUT1+ MUT2+* / *MUT1+ MUT2+*	5.7	—
DG52	*MUT1+ MUT2+* / *MUT1+ MUT2+*	5.6	130
XV175	*mut1-1 MUT2+* / *MUT1+ MUT2+*	5.9	133
XV174	*mut1-2 mut2-1* / *MUT1+ MUT2+*	7.2	183
DG7	*mut1-1 MUT2+* / *mut1-1 MUT2+*	104.4	437
DG10	*mut1-2 mut2-1* / *mut1-2 mut2-1*	114.8	375
DG8	*mut1-1 MUT2+* / *mut1-2 mut2-1*	141.3	467
DG17	*mut1-2 mut2-1* / *mut1-1 MUT2+*	109.9	396
DG44	*mut1-2 MUT2+* / *mut1-2 MUT2+*	125.9	746
DG46	*MUT1+ mut2-1* / *MUT1+ mut2-1*	22.4	109
DG56	*mut1-1 MUT2+* / *mut1-2 MUT2+*	105.1	403
DG21	*MUT1+ mut2-1* / *mut1-1 MUT2+*	6.6	156

It can be seen in Table 3 that the spontaneous mutation rate for the diploid controls in these experiments is about twice that for the haploid controls shown in Table 4. The mutator mutants are clearly recessive and their mutation rates when the alleles are homozygous in diploids are also higher than in haploids.

The qualitative effect of the mutator alleles in homozygous diploids is similar to that in haploids, that is, *mut1-1* $\simeq$ *mut1-2* and *mut 2-1* $\simeq 18 - 27\%$ the effect of the *mut1* alleles. There is one difference between the behavior of the mutator genes in haploids and diploids; there seems to be no additive effect in diploids, perhaps even an antagonistic effect in the *mut1-2 mut2-1* combination (DG44 containing *mut1-2* homozygous has a mutation rate of 125.9 mutations/cell/generation; DG46 containing *mut2-1* homozygous is 22.4; consequently, DG10 containing both *mut1-2* and *mut2-1* homozygous should be approximately 150—it is 114.8). Clearly, more experiments should be carried out to explore this phenomenon.

TABLE 4

Spontaneous mutation rates of the ochre mutant lys1–1 *and its suppressors in haploid strains*

Genotype	Strain	Mutation rates (×10^8) *lys1–1*	Mutation rates (×10^8) *SUP*	Mutation rates (×10^8) Total	No. of revertant compartments *lys1–1*	No. of revertant compartments *SUP*	Total compts.	No. cells in non-mutant compts	Amt. of limiting lys (mg/l)
MUT+	XV185–3C	0.5	1.6	2.1*	18	59	995	1.93 × 10^6	1.0
	XV185–1D	0.4	3.1	3.5*	12	91	994	1.53 × 10^6	1.0
	XV185–6A	0.5	2.4	2.9*	—	—	—	—	1.0
	XV1687–12B	0.3	2.9	3.2	12	115	1000	2.14 × 10^6	1.0
	Average	0.4	2.5	2.9					
mut1–1	XV177–23A	0	67.0	67.0*	0	992	996	4.07 × 10^6	1.0
	XV177–23D	0	104.5	104.5	†	952	997	1.46 × 10^6	1.0
	VA-3	0	61.3	61.3	0	215	1000	1.94 × 10^5	0.1
	VA-3	–	—	71.0*	—	—	999	2.45 × 10^5	1.0
	VA-3	–	—	67.3	—	—	988	6.21 × 10^5	0.3
	VA-3	0	64.9	64.9	0	162	995	1.34 × 10^5	0.1
	Average			72.7					
mut1–2	XV181–3C	0	69.7	69.7	0	874	1000	1.46 × 10^6	1.0
	XV181–3D	–	—	65.7	—	—	—	—	0.1
	DG16–1B	–	—	78.6	†	447	999	3.85 × 10^5	0.1
	Average			71.3					
mut2–1	DG16–1A	0	13.2	13.2	0	27	999	7.55 × 10^4	0.1
	XV181–3A	–	—	21.3	‡	11	1000	2.50 × 10^4	0.1
	XV353–5D	0.6	23.7	24.3*	—	—	—	—	
	Average			19.6					
mut1–2 mut2–1	VA-105	0	104.5	104.5	0	289	998	1.54 × 10^5	0.1
	VA-105	0	144.9	144.9*	0	243	994	8.85 × 10^4	0.1

* von Borstel *et al.* 1973.
† One red sector.
‡ One red colony.

TABLE 5

Spontaneous mutation rates of the ochre mutant arg4–17 *and its suppressors in haploid strains*

Genotype	Strain	Mutation rates (×10^8) *arg4–17*	Mutation rates (×10^8) SUP	Mutation rates (×10^8) Total
MUT+	X1687–12B	.33	2.9	3.3
mut1–1	VA-3	0	63.4	63.4
	VA-3*	—	—	62.7
	Average	—	—	63.1
mut1–2 mut2–1	VA-105	0	104.5	104.5
	VA-105*	—	—	90.9
	Average	—	—	97.7

* von Borstel *et al.* 1973.

TABLE 6

Spontaneous reversion rates of the missense mutant his1–7 *in haploid strains*

Genotype	Strain	Mutation rate (×10⁸)	Number of revertants
MUT+	DG15–7D	7.6	816
MUT+	DG15–7D	9.8	159
MUT+	XV185–1D	6.1*	218
MUT+	XV185–3C	7.8*	117
MUT+	XV185–6A	7.1*	119
	Average	7.7	
mut1–1	XV177–23A	42.4*	491
mut1–1	XV177–23D	37.9*	351
	Average	40.1	
mut1–2	DG16–1D	29.2	500
mut1–2	XV181–3C	12.8*	123
	Average	21.0	
mut2–1	XV353–5D	22.8*	—
mut2–1	DG50–8B	13.9	974
	Average	18.4	

* VON BORSTEL *et al.* 1973.

Reversion of a missense mutant: The data for reversion of the missense mutant *his1–7* are shown in Table 6. Both *mut1–1* and *mut1–2* exhibit reversion rates higher than the control, but *mut1–1* appears to act more strongly than *mut1–2*. This is unlike the data obtained for reversions for *lys1–1* where *mut1–1* and *mut1–2* act similarly both with respect to mutator activity and antimutator activity. The mutator *mut2–1* also shows slight enhancement.

Reversion of a frameshift mutant: The data for reversion of a putative frameshift mutant *hom3–10* are shown in Table 7. This mutant was tested by the method of the median which is not as accurate as the 1000-compartment box test since the number of samples is smaller. The slight differences from the control values for the double mutant and the strain with *mut1–1* are not significant.

TABLE 7

Spontaneous reversion rates for the frameshift mutant hom3–10 *in haploids*

Genotype	Strain	Reversion rate (×10⁸)
MUT1+ MUT2+	DG15–7D	1.0
MUT1+ MUT2+	DG15–7D	1.2
	Average	1.1
mut1–1 MUT2+	DG31–1B	0.4
mut1–2 mut2–1	DG16–2D	1.7
mut1–2 mut2–1	DG16–2D	1.9
	Average	1.8

TABLE 8

Spontaneous mutation rates for canavanine sensitivity to resistance in haploid strains

Genotype	Strain	Mutation rate ($\times 10^8$)
MUT+	XV169-1A	15.4
mut1-1	DG31-1B	19.8
mut2-1	DG50-4C	23.7

Forward mutation rates for drug resistance: When the mutator stocks were being isolated one of the tests for mutator activity was the appearance of parafluorophenylalanine-resistant mutants where 2.5×10^6 cells were spread on a plate containing 250 μg/ml of parafluorophenylalanine (VON BORSTEL *et al.* 1973). Stocks VA-3 and VA-105 were near the control level in their production of resistant mutants. Therefore one experiment was performed using the appearance of canavanine-resistant mutants as a criterion of mutation. Canavanine-resistant mutations map at a single locus and all mutants are recessive. The rates as established by the method of the median of LEA and COULSON (1949) are shown in Table 8. It can be seen that neither *mut1-1* nor *mut2-1* differ markedly from the control in their mutation rates to canavanine resistance.

DISCUSSION

Two stocks exhibiting high spontaneous mutation rates, VA-3 and VA-105, are described. Experiments have shown the existence of two linked mutator loci in these stocks, with two alleles at one locus and one at the other. All three mutator mutants are recessive.

In general, there is excellent agreement for the behavior of *mut1-1* and *mut1-2* with respect to two different suppressible mutations, *lys1-1* and *arg4-17*. The effect of the mutator at the second locus, *mut2-1*, on the reversion rates of these two ochre mutations is not as great. Enhanced reversion rates for a missense mutation, *his1-7*, are observed for both mutator loci. Measurement of the effects of these mutations on the reversion rates of a putative frameshift mutation and a forward mutation system to canavanine resistance was less informative because the method used in these experiments for determining the rates was less sensitive. It appears that neither *mut1-1* nor *mut2-1* have a significant effect on the induction of canavanine-resistant mutations.

The primary effect of the two *mut1* alleles is a marked enhancement of the *SUP* loci mutations and a depression in the rate of revertants of the ochre mutant itself. There is also a slight effect on the reversion of a missense mutation. These mutators are relatively specific in their action—the only marked effect is on the suppressors. It has been demonstrated that *mut1* is general in its mutator activity on the class I suppressor loci and not restricted to one suppressor locus (QUAH, TEH and VON BORSTEL, 1975).

Since locus reversions appear to be reduced in the presence of the *mut1-1* and *mut1-2* genes, it is likely that transversion is not the primary mechanism for

mutator action for these mutator alleles (for reversion of an ochre mutant by replacement with an amino acid there are six possible transversions and one possible transition). The data with the missense mutation (*his1–7*), affected by *mut1–1* and *mut1–2* and the lack of effect with the presumptive frameshift mutation, *hom3–10*, lends support for transition as the possible mechanism. On the other hand, it is possible that *mut1* may be responsible preferentially for a specific sequence of bases in the Class I suppressor loci.

Finally, the lack of mutator activity for appearance of drug resistance should be considered. It is possible that the ineffectiveness of *mut1–1* can be explained because it has both mutator activity and antimutator activity, depending on which type of molecular lesion is being assayed. The mutator *mut2–1* seems to be more general in its action, affecting most of the specific reversion assays although not to the same degree. It is possible that *mut2–1* is a simultaneous antimutator for a molecular lesion which is not available to us. If this is the case, it may be a rule that some of the loci designated as mutators may have both mutator and antimutator activity. Thus, the spectrum of spontaneous mutations could shift markedly from the wild type for some of the mutator strains, but the overall spontaneous mutation rate might remain essentially the same as the wild type. Again, it is also possible that the mutator activity may reside in specific base sequences in the target gene, and lack of mutator activity for forward mutations could be because these specific base sequences are lacking in the target gene.

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