THE ISOLATION OF MMS- AND HISTIDINE-SENSITIVE MUTANTS IN *NEUROSPORA CRASSA*

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Manuscript received June 7, 1980
Revised copy received December 16, 1980

ABSTRACT

A simple method of replica plating has been used to isolate mutants of *Neurospora crassa* that have increased sensitivity to methyl methanesulfonate (MMS) and/or to histidine. Twelve mutants with increased sensitivity to MMS and one mutant with increased sensitivity to histidine showed Mendelian segregation of the mutant phenotypes. Three mutants were mapped to loci not previously associated with MMS sensitivity. Two others were allelic to the UV- and MMS-sensitive mutant, *mei-3*. Survival curves indicate that conidia (mutant or wild-type) survive on much higher concentrations of MMS at 25° than at 37°. In contrast, mycelial growth is more resistant to MMS at 37°. The possibility of qualitatively different repair processes at these two temperatures is discussed.

THE control of DNA repair is the subject of intensive investigation in both prokaryotic and eukaryotic organisms. The analysis of DNA repair functions by a combined genetic and biochemical approach has been quite effective in bacteria (see HANAWALT *et al.* 1979). In most eukaryotes, the application of this approach is limited, primarily due to the lack of a large number of relevant mutants. Only in *Saccharomyces cerevisiae* is a large group of repair-defective mutants available; these mutants, representing about 55 loci, have increased sensitivity to radiation and/or chemical mutagens (COX and PARRY 1968; GAME and MORTIMER 1974; PRAKASH and PRAKASH 1977).

A limited number of mutants with increased sensitivity to UV has been isolated in the filamentous ascomycete *Neurospora crassa* (see SCHROEDER 1975). This paper reports the isolation and partial characterization of Neurospora mutants with increased sensitivity to the alkylating agent methyl methanesulfonate (MMS). Since histidine sensitivity has been associated with several UV-sensitive mutants (NEWMEYER, SCHROEDER and GALEAZZI 1978), mutants with increased sensitivity to histidine were also selected. To obtain mutants defective in a variety of repair functions normally present in actively growing cells, the selection procedure monitored sensitivity of actively growing mycelia, rather than asexual conidia.

Twelve MMS-sensitive mutants and one histidine-sensitive mutant were obtained. Five of the MMS-sensitive mutants were mapped to specific loci. Two of these mutants are allelic to the previously isolated *mei-3*. Survival curves of

seven mutants show that these mutants should be useful in the study of DNA repair in *N. crassa*.

MATERIALS AND METHODS

Strains: The strains used for mutant isolation and mapping are shown in Table 1. The mutagen-sensitive mutants *uvs-2* (no allele number) IV R, *uvs-3* (ALS 11) IV L, *uvs-4* (ALS 12) III R, *uvs-5* (ALS 13) III R, *uvs-6* (ALS 35) IR, and *upr-1* (no allele number) IL and the meiotic mutant *mei-3* (N 289) IC, which were obtained from the Fungal Genetics Stock Center, were crossed with two strains of opposite mating type containing *pan-1* or *inl* (FGSC 1438, 1453, 2657, 2658). In each case, mutant ascospore cultures carrying one auxotrophic marker (*pan-1* or *inl*) and *Cde* compatibility genotype were obtained for use in complementation tests.

Media: The medium used to replica-plate colonies is a modified plating medium (Davis and deSerres 1970). All media contained 1% L-sorbose. Instead of 0.05% fructose and 0.05% D-glucose as carbon sources, only 0.01% D-glucose was used. This slows down the growth of replicating colonies, thereby allowing easier recognition of mutants with increase sensitivity to MMS. Initially, all plating media contained only 0.01% D-glucose. However, when ascospores were plated on such media, only 5–15% of the germinated ascospores would form colonies; therefore, 0.05% fructose, 0.05% glucose medium was used to plate ascospores. In such cases, the colonies were still replica-plated onto 0.01% D-glucose medium (see next section). The media contained the appropriate supplements to allow growth; in addition, MMS (0.01% unless otherwise stated), and histidine (500 mg/l) was added to the low glucose (MMS + his) medium. The MMS was added to autoclaved medium that had been allowed to cool to about 60°, an the plates were used within 24 hr. All other types of media have been described previously (Davis and deSerres 1970).

Mutant isolation: Conidia from a 7-day-old culture of the *A sn cr-1*; *al-3*, *inl* strain (FGSC 2464) were mutagenized with 25 mm nitrosoguanidine (pH 7.0) for 2, 4 or 6 hr (Malling and deSerres 1970). These conidia were plated by spreading 0.8 ml of conidial suspension, containing about 75–150 viable conidia, on low glucose medium that had been allowed to dry for 4–5 days at room temperature. The resulting colonies were replica-plated, using a modification of the method described by Littlewood and Munkres (1972), by allowing the mycelia to grow into a 7.0 cm filter paper (FP) disc (Whatman #1), and transferring this disc after 3 days to a new plate containing low glucose. Mycelia were allowed to grow into each replica plate at 15° for about 24 hr. The first replica was used as a control for replicating ability. The disc was then transferred

TABLE 1

Strains used in mutant isolation, mapping and complementation studies

Strain no.	Genotype* (allele number)	Linkage group
FGSC2464	*A*,*sn*(C136),*cr-1*(B123);*al-3*(RP100),*inl*(83201t)	IC,R;VR,R
FGSC1438	*a*;*inl*(37401)	VR
FGSC1453	*A*;*inl*(37401)	VR
FGSC2657	*a*,*al-2*(15300);*pan-1*(5531)	IR;VR
FGSC2658	*A*,*al-2*(15300);*pan-1*(5531)	IR;VR
FGSC2282	*un-5*(b39t),*A*,*al-2*(15300),*arg-13*(RU3)	IL,R,R
FGSC1290	*A*;*cys3*(P22),*arg-5*(27947)	IIL,C
FGSC2125	*A*;*acr-2*(KH5),*trp-1*(10575),*dow*(P616)	IIIC,R,R
FGSC161	*A*;*pdx-1*(27803),*pan-1*(5531),*pyr-2*(28502)	IVC,R
FGSC1535	*A*;*lys-1*(33933),*inl*(37401),*his-6*(Y152M105)	VC,R,R
FGSC210	*A*;*asco*(37402),*trp-2*(75001)	VIL,R
FGSC157	*A*;*nic-3*(Y31881),*wc*(P829),*arg-10*(B317)	VII,R,R

* The mating-type locus (*A*/*a*) is located on LGIL. Strains FGSC 1438, 1453, 2657 and 2658 have the *Cde het* genotype.

to medium containing 0.01% MMS and 500 mg/l histidine and incubated again at 15° for 24 hr. After removal of the FP disc, each plate was incubated at 37° for 2–4 days, after which the two replica plates were scored.

The concentration of MMS has been determined by testing the ability of colonies to replica-plate on medium containing different concentrations of MMS. The concentration of histidine is identical to that used previously to inhibit histidine-sensitive mutants (NEWMEYER, SCHROEDER and GALEAZZI 1978). Replica-plating of wild-type colonies onto 0.01% MMS resulted in dense colonies, but replica-plating onto 0.02% MMS produced very sparse colonies. The addition of histidine did not alter these results.

To confirm the mutant phenotype, the mutant colonies were transferred to solid medium by means of an applicator stick and replica-plated as before. A total of 30 strains still appeared sensitive after testing 79 presumptive mutants. Conidia from each of these strains were plated on low glucose medium and again replica-plated. Individual conidial isolates were then used in a cross with strain *a al-2*; *pan-1* (FGSC 2657). The resulting ascospores were plated onto regular plating medium (0.05% fructose; 0.05% glucose; see Media), and then the germinating ascospore colonies were replica-plated onto medium containing MMS and/or histidine. Only those mutants that segregated approximately equal numbers of resistant and sensitive colonies were tested further. The mutants were assigned the locus designation *mus* (for mutagen sensitivity) or *shi* (if only histidine-sensitive) and isolation numbers SC1, 2, etc.

Complementation tests: The forced heterokaryons used in complementation tests were of genotype *a* (or *A*) *pan-1*; *mutant-1 Cde* and *a* (or *A*) *inl*; *mutant-2 Cde*. Sensitivity of these heterokaryons was tested on minimal low glucose medium by the standard replica-plating method, using FP discs. The failure to isolate appropriate *Cde* tester strains for some mutants is one major reason that the complementation analysis is incomplete.

Crosses and other procedures: Crosses were carried out on solid slants of synthetic crossing medium. Fertilization was effected 7 days after inoculation of the female parent by adding a fresh conidial suspension of the male parent. When used in a cross, *cr-1* strains were always used as the male parent since they act very poorly or not at all as females. Replica-plating with velveteen and other routine procedures have been described previously (LEDERBERG and LEDERBERG 1952; DAVIS and DESERRES 1970).

Mapping: (1) Since the first cross of each mutant contained markers on LG I [*sn*, *cr*, *al-2* and mating type (*A/a*)], LG IV (*pan-1*), and LG V (*al-3* and *inl*), this cross was used to detect linkage to these groups. Linkage of the remaining mutants and more accurate mapping were achieved with crosses to the multiply marked tester strains shown in Table 1.

(2) The seven tester strains, each of which carries several auxotrophic markers spanning a single linkage group, were used to assign each mutant to a particular linkage group and to determine its approximate location on that linkage group. First, ascospores from each of the 7 crosses were spread onto minimal medium (or minimal + acroflavin to test for linkage to *acr* on LG III, and replica-plated onto (MMS + his) medium to test for the sensitive genotype. Linkage was indicated when significantly more than 50% of the colonies were sensitive. Second, a more precise mapping of the mutant on the designated linkage group was accomplished by plating the ascospores on medium supplemented with one or more requirements. Further routine mapping procedures were as described previously (see DAVIS and DESERRES 1970).

MMS treatment (for survival curves): Seven-day-old conidial cultures were suspended in 0.05 M potassium phosphate buffer, pH 7.0. These conidial suspensions were either diluted and plated onto different concentrations of redistilled MMS (generously donated by L. PRAKASH) or incubated for up to 60 min at 30° with 0.5% MMS followed by dilution and plating onto minimal plating medium (PRAKASH and PRAKASH 1977).

RESULTS

Isolation of mutants: Over 9500 colonies, produced by the germination of mutagenized conidia, were examined for increased sensitivity to MMS and/or histidine, using the method of replica-plating described in MATERIALS AND METH-

ODS. Of the 79 prospective sensitive colonies retested, 30 were sensitive to MMS + histidine. These mutants were tentatively designated *mus-(SC1)* through *mus-(SC30)*. In crosses with wild-type strain *mus*$^+$; *pan-1*; *al-2 a*, 14 of these mutants segregated in a Mendelian fashion (see Table 2); only these were tested further. In at least one case, *mus-(SC13)*, the mutant effect was heat sensitive, *i.e.*, sensitive at 37° but not at 25°.

Since the replica-plating procedure used to isolate the mutants involved consecutive transfer and a 24-hr incubation at 15° of filter paper on (minimal) and (MMS + his) media, the failure to grow on the (MMS + his) medium could be caused by reduced growth potential of colonies after the initial 24-hr period at 15°. Therefore, all mutants were replica-plated consecutively onto minimal medium. In all cases, except for mutant SC2, normal growth resulted on both replica plates. Thus, SC2 appears to be a cold-sensitive mutant that loses viability during prolonged incubation at the restrictive temperature (15°). This mutant also grows very poorly at 25°, but quite well at 37°.

Sensitivity of the remaining 13 mutants to MMS and to histidine: Using the FP replica-plating procedure, conidia from each of the original mutant strains were separately tested for MMS- and histidine-sensitivity. Three classes of mutants were distinguished on the basis of the phenotypes of the colonies (Table 2). Mutants *mus-(SC9)*, *mus-(SC25)* and *mus-(SC28)* were sensitive to both MMS and histidine; mutants *mus-(SC1)*, *mus-(SC3)*, *mus-(SC10)*, *mus-(SC13)*, *mus-(SC15)*, *mus-(SC17)*, *mus-(SC20)*, *mus-(SC26)* and *mus-(SC29)* to MMS alone, and mutant *shi-(SC14)* to histidine alone. In addition, no significant differences were detected whether these mutants were tested at 25° or at 37°.

TABLE 2

Sensitivity to MMS and histidine of mycelia from 14 mutants and segregation of the mutants in crosses

Mutant allele	Sensitivity* of mutants isolates to		Progeny from the cross mutant (m) × wild type (m^+)		
	MMS	HIST.	% Black ascospores	No. of isolates of genotype m	m^+
SC1	S	+	90–100	18	17
SC2	+	+	90–100	24	63
SC3	S	+	90–100	33	59
SC9	S	S	90–100	13	13
SC10	S	+	30–40	11	14
SC13	S	+	90–100	20	19
SC14	+	S	90–100	24	27
SC15	S	+	90–100	47	25
SC17	S	+	90–100	23	41
SC20	S	+	30–40	25	38
SC25	S	S	90–100	16	22
SC26	S	+	20–30	10	9
SC28	S	S	90–100	31	54
SC29	S	+	90–100	18	46

* S = sensitive; + = resistant.

Sensitivity of conidia from mutant strains: Mutants with increased sensitivity to MMS and to histidine have been detected by a decreased ability of mycelial fragments, which were transferred by a 24-hr incubation of filter paper discs at 15°, to produce dense colonies on medium supplemented with MMS or histidine. To determine whether conidia of these mutants were similarly incapable of producing dense colonies on these inhibitory media, conidia of representative *cr**; *mus* (or *shi*) ascospore isolates were replica-plated, using velveteen, on medium supplemented with both MMS and histidine. The replica plates were incubated at 25° and 37°, and the colonies were scored after 3 to 4 days (Table 3). Using this method, six mutants, *mus-(SC3)*, *mus-(SC13)*, *mus-(SC15)*, *mus-(SC25)*, *mus(SC28)*, and *mus-(SC29)*, appeared more sensitive than wild type. In contrast, *mus-(SC1)*, *shi-(SC14)*, *mus-(SC17)* and *mus-(SC26)* were not detectably more sensitive than wild type. The MMS sensitivity of *mus-(SC1)* and *mus-(SC17)* and the histidine-sensitivity of *shi-(SC14)* may be expressed only after incubation at 15°. This need for pre-incubation at 15° was confirmed by the finding that replica-plating with incubation of filter paper at 37° (instead of 15°) failed to produce the mutant phenotype in these three cases. Of these three mutants, only *mus-(SC17)* appeared cold-sensitive for growth on minimal medium.

Survival curves of MMS-sensitive mutants: The degree of sensitivity of the six mutants with sensitive conidia (see previous section) and *mus-(SC10)* was assessed by their relative sensitivities on media containing various concentrations of MMS and by survival curves after treatment with MMS. First, after spreading conidia of representative mutant ascospore isolates on media with different

TABLE 3

*Sensitivity to MMS + histidine of conidia from mutant ascospore isolates**, *determined by replica plating with velveteen*

Allele no.	Sensitivity† to MMS + histidine at	
	37°	25°
SC1	+	+
SC3	S	S
SC13	S	+
SC14	+	+
SC15	S	+
SC17	+	+
SC25	S	S
SC26	+	+
SC28	+	S
SC29	S	S
ORA (wild-type)	+	+

* Mutants *mus-(SC9)* and *mus-(SC20)* were not tested since no *cr* ascospore isolates were available, and the *mus-(SC10)* isolate used did not replicate, probably due to a lack of conidia.
† Strains were considered sensitive(S) if, 3–4 days after replica-plating, a colony was produced on minimal but no or very little growth resulted on medium supplemented with MMS + histidine.

* The presence of *cr* allows replica-plating of conidia; *mus-(SC9)* and *mus-(SC20)* were not tested because no *cr; mus* ascospore isolates of these mutants were available.

concentrations of MMS, they were incubated at 25° and 37°. For all mutants, as well as wild-type ORA, two qualitatively different patterns of survival resulted at these two temperatures. Whereas typical survival curves were obtained after incubation at 37° (Figure 1), incubation at 25° failed to produce such curves. Instead, a particular threshold concentration existed above which all colonies grew well and below which all colonies grew poorly or not at all. This is in contrast to colonies produced at 37°, which were dense, whether plated on medium lacking MMS or with 0.03% MMS (the highest concentration used). At 25°, the threshold concentrations were 0.002% to 0.005% for *mus-(SC3)* and *mus-(SC10)*, 0.005% to 0.01% for *mus-(SC15)*, *mus-(SC25)* and *mus-(SC29)*, and 0.01% to 0.02% for *mus-(SC13)*, *mus-(SC28)* and wild-type ORA. At 37°, these seven mutants could be subdivided into two classes, four [*mus-(SC10)*, *mus-(SC25)* and *mus-(SC29)*] that were very sensitive and three [*mus-(SC3)*, *mus-(SC13)* and *mus-(SC28)*] that had an intermediate sensitivity (Figure 1).

Second, survival curves of conidia after MMS treatment were obtained with

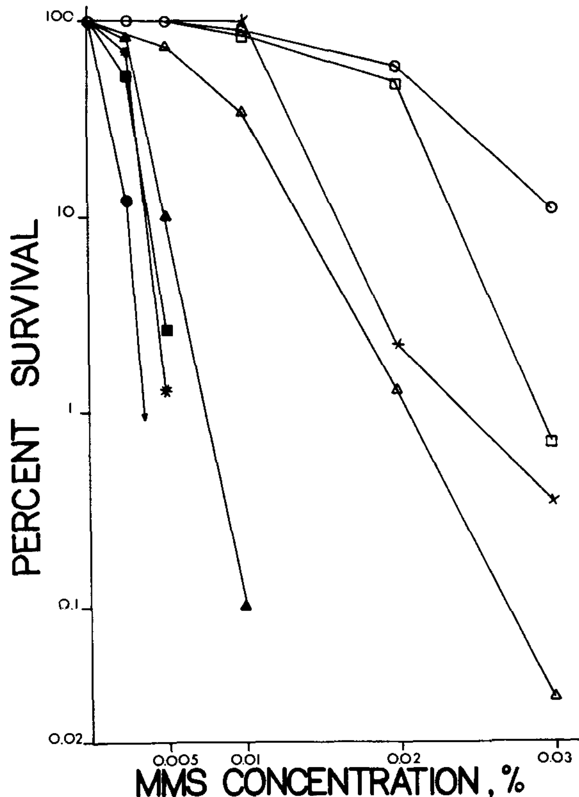


FIGURE 1.—Survival of conidia from 7 MMS-sensitive mutants and wild-type ORA when plated on various concentrations of MMS and incubated at 37°. The strains are ORA (O), and an ascospore isolate of each of the MMS-sensitive mutants *mus-(SC13)* (□), *mus-(SC3)* (X), *mus-(SC28)* (△), *mus-(SC15)* (▲), *mus-(SC25)* (■), *mus-(SC10)* (*), and *mus-(SC29)* (●). Repeats of one or more isolate of each mutant produced similar survival curves.

incubation at 25° and 37° (Figure 2–9). A typical curve with a shoulder was obtained for wild-type ORA. Mutants *mus-(SC10)*, *mus-(SC25)* and *mus-(SC29)* were very sensitive at both temperatures (Figures 4, 8, 9), *mus-(SC13)* was sensitive at 37° but not at 25° (Figure 5), *mus-(SC15)* was only slightly sensitive, primarily due to the lack of a shoulder (Figure 6), *mus-(SC28)* was not sensitive (Figure 7) and *mus-(SC3)* was resistant to MMS, especially at 25°. In general, both mutants and wild type were more sensitive at 37° than at 25°.

Linkage studies: The initial crosses between mutant (sensitive) and wild type were used to locate mutants to Linkage groups I, IV, and V. Thus, *mus-(SC25)* and *mus-(SC29)* were mapped on LG I between the mating-type locus (*A/a*) and *cr-1*, *mus-(SC28)* on LGIR distal to *al-2* and *mus-(SC15)* and *mus-(SC17)* on LG V (Table 4). More detailed mapping of *mus-(SC15)* and *mus-(SC17)* located *mus-(SC15)* 10.2 mu to the left of *inl* and *mus-(SC17)* 27.0 mu to the left of *inl* (Table 4). Preliminary data suggest that *mus-(SC3)* is located on LG VI near *asco*. Mutant *mus-(SC10)* showed linkage to three different linkage groups: LG II (near *arg-5*, no recombinants among 32 spores examined), LG III (right of *trp-1*, 1 recombinant among 66 spores analyzed) and LG VI (left of *ad-1*, 10 recombinants among 53 spores analyzed).

MMS-sensitivity of mus-(SC26) *and the role of uracil*: Two crosses between

TABLE 4

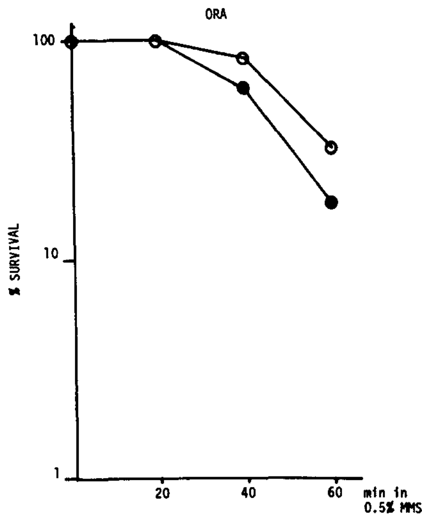
Mapping of mus *mutants*

Linkage to LG	Mutant allele	Zygote genotype and recombination %	Fraction of recombinants		
			A/a–mus	*In region cr-1–mus*	*al-2–mus*
I*	*mus-(SC25)*	*A mus cr-1 al-2* / *a* + + + (3.3, 6.7)	1/30	2/30	5/19
	mus-(SC29)	*A mus cr-1 al-2* / *a* + + + (9.4, 18.7)	3/32	6/32	4/14
	mus-(SC28)	*A cr-1 al-2 mus* / *a* + + + (17.6)	15/40	11/40	3/17
			On medium supplement with		
			lys	*his*	
V†	*mus-(SC15)*	+ *mus* + + / *lys* + *inl his-6* (10.2)	5/49	0/112	
	mus-(SC17)	+ *mus* + + / *lys* + *inl his-6* (27.0)	10/37	0/136	

* Ascospore isolates were separately picked and their markers tested; *sn*, between *A/a* and *cr-1*, was not scored. Since the closely linked *al-3* and *inl* (LG V) were also present in this cross, linkage to *al-2* could be estimated in inl^+ progeny only.

† The distance between *mus* and *inl* was determined by the replica-plating method described in MATERIALS AND METHODS: on medium lacking inositol, only parental *mus* inl^+ and recombinant mus^+ inl^+ colonies grew; whether *mus* was to the left or right of *inl* was inferred from platings on media with different supplements: the lack of colonies on histidine-supplemented medium places both *mus-(SC15)* and *mus-(SC17)* to the left of *inl*.

mus-(SC26) and *A*; *pdx-1 pan-1 pyr-2* (FGSC 161) produced nearly 50% sensitive colonies (9/17 and 18/35) on medium supplemented with pyridoxine (pdx) and pantothenic acid (pan), but no sensitive colonies (0/24 and 0/28) on medium supplemented with uracil (pyr) and pantothenic acid. It is plausible that the mu-



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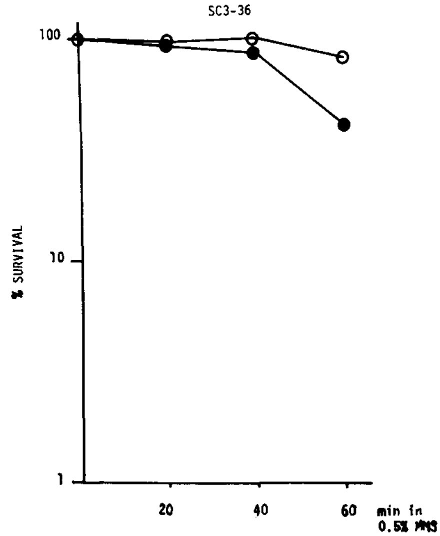

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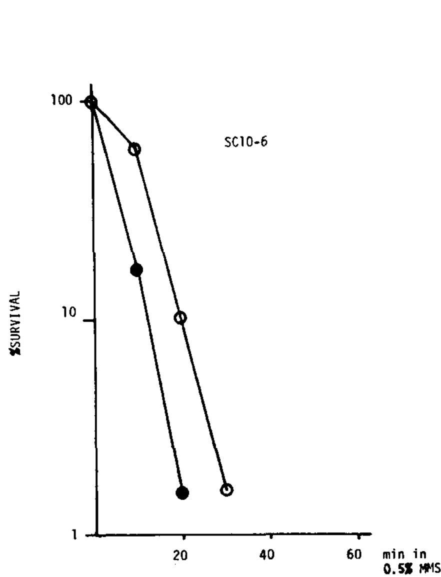

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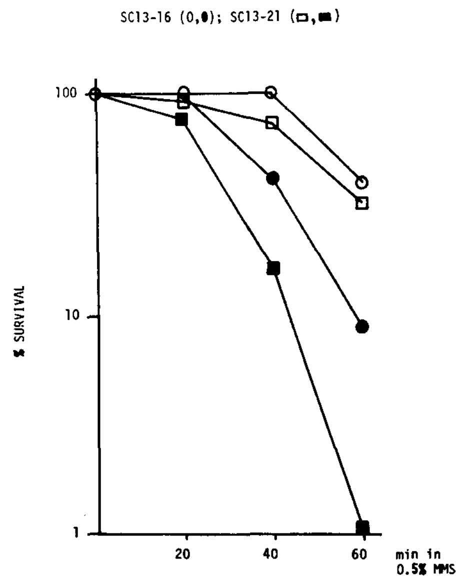

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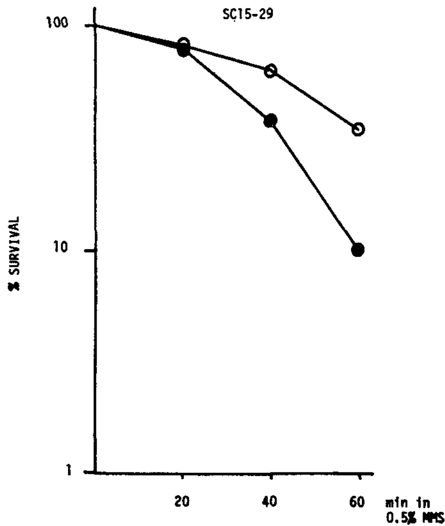
FIGURES 2–9.—Survival curves of conidia from 7 MMS-sensitive mutants and wild-type ORA after treatment with 0.5% MMS; plates were incubated at 25° (O, □) and at 37° (●, ■). The strains are ORA (Figure 2), one ascospore isolate of each of the mutants *mus-(SC3)* (Figure

tant phenotype may be supplemented by uracil. To determine whether *mus-(SC26)* is a leaky uracil-requiring mutant, growth rates of *mus* and *mus*$^+$ ascospore isolates from a heterozygous cross were tested on media with and without uracil. Whereas all nine *mus*$^+$ isolates showed no difference on the two types of



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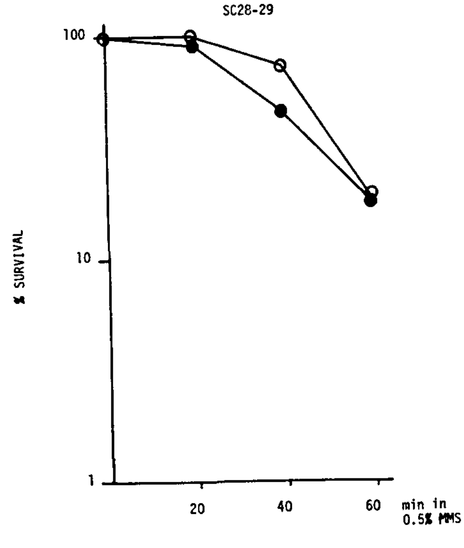

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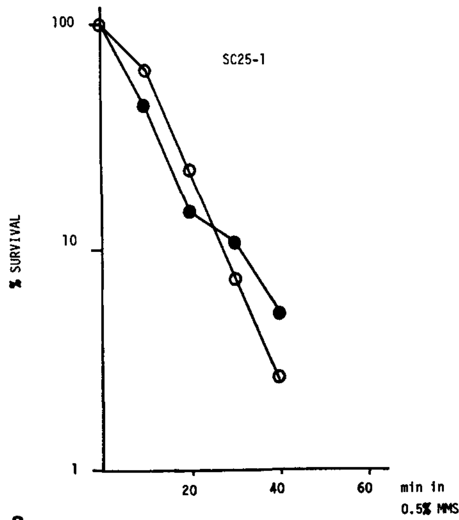

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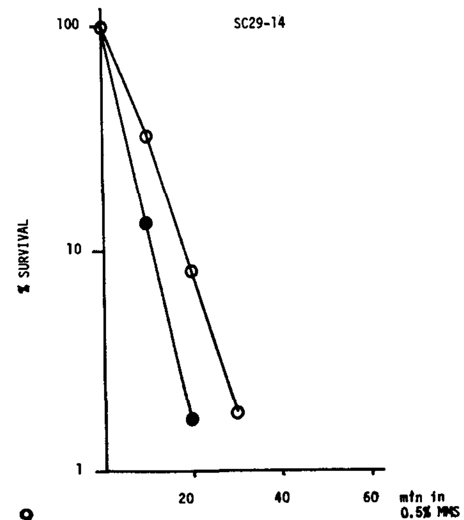

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3), *mus-(SC10)* (Figure 4), *mus-(SC15)*, (Figure 6), *mus-(SC28)* (Figure 7), *mus-(SC25)* (Figure 8), *mus-(SC29)* (Figure 9) and two ascospore isolates of *mus-(SC13)* (Figure 5). These curves were repeated with essentially identical results.

media, growth of the eight *mus* mutants was either inhibited (3) or stimulated (5) by uracil. From these data, it is concluded that the *mus-(SC26)* mutant was not pyrimidine-requiring (uracil), but that a change in the pyrimidine pool may cause the MMS-sensitivity of this mutant.

Fertility of crosses between the mutants and wild-type strains: The original mutants, *mus-(SC10)*, *mus-(SC20)* and *mus-(SC26)*, resulted in significant ascospore abortion when crossed with a wild-type strain of opposite mating type (see Table 2). The sensitive and abortion phenotypes could be separated in the case of *mus-(SC26)*. The co-segregation of these two phenotypes has not been established in the case of *mus-(SC20)* because of difficulties encountered in scoring MMS-sensitivity of ascospore isolates. However, *mus-(SC10)* is very sensitive, and the two phenotypes co-segregated in 24/25 ascospore isolates. A cross involving the remaining isolate appeared to be barren, but produced clusters of black ascospores in a delayed fashion. This may have been due to reversion or contamination. Thus, MMS-sensitivity of *mus-(SC10)* co-segregates with a dominant ascospore abortion factor or is very closely linked to it. The ascospore abortion is apparently due to a multiple translocation involving LG II, LG III and LG VI (see section on Linkage studies). In addition, *mus-(SC10)* also co-segregates with female sterility.

Analysis of previously isolated mutants sensitive to MMS: The *uvs-2*, *uvs-3*, *uvs-6* and *upr-1* mutants were previously found to be sensitive to MMS (FRASER 1979). To compare these mutants with the mutants isolated during this study, the MMS sensitivity of these and other mutants was tested using the FP replica-plating procedure. Segregation of sensitive and resistant colonies from heterozygous crosses was observed for *uvs-2*, *uvs-3*, *uvs-4*, *uvs-6*, *upr-1* and *mei-3*, but not for *uvs-5* and *nuc-2*. The phenotype of *upr-1* colonies could not always be scored with confidence.

Allelism: Allelism of any two mutants is usually established by their inability to complement in a heterokaryon. Such complementation data could not be obtained from all combinations of mutants because of difficulties in obtaining heterokaryon-compatible strains for all mutants. In addition, results from some tests became meaningless because of scoring difficulties (*i.e.*, positive tests on MMS-containing medium) of some homokaryotic tester strains. Consequently, this analysis has provided data regarding only a subgroup of mutants: mutants *uvs-2*, *uvs-3*, *uvs-4*, *uvs-6*, *mus-(SC10)*, *mus-(SC26)* and *mus-(SC28)* complemented each other; they also complemented *mei-3*, *mus-(SC25)* and *mus-(SC29)*, but both *mus-(SC25)* and *mus-(SC29)* failed to complement *mei-3*. Of these mutants, only *mus-(SC26)* has not been mapped yet. Consequently, allelism of this mutant to *mus-(SC3)*, which is apparently located on LGVI, has not been ruled out. Similarly, *mus-(SC3)* and *mus-(SC10)* could be allelic, since *mus-(SC10)* is linked to LG II, LG III and LG VI.

Allelism of mei-3 *and the newly obtained* mus-(SC25) *and* mus-(SC29): Three pieces of information suggest that *mus-(SC25)* and *mus-(SC29)* are allelic to the previously isolated *mei-3*. (1) All three mutants are quite sensitive to MMS [the sensitivity to histidine has not yet been sufficiently studied for *mus-(SC25)* and

mus-(SC29)], and both *mus-(SC25)* and *mus-(SC29)* fail to complement *mei-3* to relieve its sensitivity to MMS. (2) Both *mus-(SC25)* and *mus-(SC29)* were mapped in the same region as *mei-3*, near the centromere of LG1 (see Table 4; NEWMEYER and GALEAZZI 1978). (3) Crosses of *mus-(SC25)*, *mus-(SC29)* and *mei-3* to wild-type strains were quite fertile. However, crosses homozygous for each of these mutants were infertile and produced barren perithecia. In addition, all interallelic crosses involving these three mutants were similarly infertile.

DISCUSSION

Using a replica-plating procedure with Neurospora that allowed the detection of increased MMS- and histidine-sensitivity of actively growing mycelia, 12 mutants with increased MMS sensitivity (*mus*) and one with increased histidine sensitivity (*shi*) have been isolated. Allelism tests and mapping of some of these mutants indicate that *mus-(SC3)*, *mus-(SC15)*, *mus-(SC17)* and *mus-(SC28)* represent new loci distinct from previously obtained mutants with increased MMS sensitivity, *uvs-2*, *uvs-3*, *uvs-6*, *upr-1* (FRASER 1979), *uvs-4* and *mei-3* (see RESULTS). Two mutants, *mus-(SC25)* and *mus-(SC29)*, were shown to be allelic to *mei-3*.

When mutant conidia were tested for survival on MMS-containing media, qualitatively different survival curves were obtained at 25° and 37°. This different pattern is exemplified well by *mus-(SC29)*. At 37°, less than 0.1% of conidia produce colonies on 0.005% MMS; whereas, at 25° all conidia grow well on 0.005% MMS, but 100% very poor-growing colonies were apparent on 0.01% MMS. Both mutants and wild type had a similar qualitative difference. Since all colonies produced at 37° grow well irrespective of the concentration of MMS in the medium, it is evident that actively growing mycelia of Neurospora are much more resistant to MMS at 37° than at 25°. In contrast, both mutant and wild-type conidia appear to be more resistant to the lethal damage of MMS at 25°. It is presently not clear whether the differential sensitivities observed are due to differential uptake of MMS, induction of damage to DNA or DNA repair capacities.

Similar observations have previously shown that sensitivity to histidine of some UV-sensitive mutants was preferentially expressed at 37° (NEWMEYER, SCHROEDER and GALEAZZI 1978). It has also been observed that only UV-sensitive mutants that are simultaneously recessive meiotic mutants (*i.e.*, *uvs-3*, *uvs-4*, *uvs-5*, *uvs-6*, *mei-3*) are sensitive to histidine. In this respect, it is of interest to note that the sexual cycle, including meiois, in Neurospora functions only at temperatures below 28–30°. These observations, together with the data on the qualitatively different survival of conidia on MMS media at the two temperatures, are suggestive of qualitative differences in DNA repair and associated processes at 25° and 37°. The continued isolation and characterization, both genetic and biochemical, of MMS- and histidine-sensitive mutants should clarify whether such differences in DNA repair do exist.

Preliminary characterization of some MMS-sensitive mutants has revealed several types. First, *mus-(SC10)*, *mus-(SC25)* and *mus-(SC29)* are very sensitive

to MMS at both 25° and 37°, whether growth on MMS media or survival after MMS treatment was the criterion. Second, *mus-(SC15)* was very sensitive when grown on MMS media, but the survival curve for this mutant after MMS treatment differed from wild type only in missing the characteristic shoulder. This mutant appears to be defective in a function needed mainly for DNA repair in replicating cells, but not in conidia. This mutant resembles *mms2–1*, *mms10–1* and *mms22–1* of yeast (PRAKASH and PRAKASH 1977). Mutant *mus-(SC28)* is similar to *mus-(SC15)*, except that it is not as sensitive on MMS media and has a wild-type survival curve after MMS treatment. Third, *mus-(SC13)* appears sensitive to MMS only at 37°. Fourth, the extreme sensitivity of *mus-(SC3)* on MMS media and its increased resistance after MMS treatment may well be attributed to the slow growth rate of this mutant. The interaction of a lengthened cell cycle and DNA repair would be expected to produce increased resistance to MMS. Finally, *mus-(SC1)* and *mus-(SC17)* appear to require pre-incubation at 15° before MMS sensitivity is expressed. It is not clear whether cold-sensitive growth is the cause of their subsequent inability to grow on MMS.

The MMS-sensitivity of *mus-(SC26)* can apparently be overcome with uracil. Initial studies indicate that *mus-(SC26)*, though not a pyrimidine-requiring mutant, may affect the pyrimidine pool. Even though pyrimidine-requiring mutants of Neurospora are apparently not UV-sensitive, analogous mutants in *Ustilago maydis* are sensitive to UV, γ rays and nitrosoguanidine (MOORE 1975 a, b) and growth of the UV-sensitive mutant *rad1–1* of *Saccharomyces cerevisiae* appears to be stimulated by pyrimidines (NAKAI and MATSUMOTO 1967). Analysis of *mus-(SC26)* of Neurospora may possibly help clarify why pyrimidine-requiring mutants of Ustilago are sensitive to DNA-damaging agents, but those of yeast and Neurospora are not.

In future studies we will pursue the isolation of more MMS-sensitive mutants, concentrating on temperature-sensitive types, and the analysis of all mutants for possible defects on the molecular level, using *in vivo* and *in vitro* bioassays.

We wish to thank L. PRAKASH for the generous donation of redistilled MMS. This work was supported by a grant from the U.S. Department of Energy (Contract No. E. P. 78-S-09-1071).

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Corresponding editor: C. W. SLAYMAN