

## Temperature-sensitive mutants

D. Stadler

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### Abstract

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Thirty-four temperature-sensitive mutants which were isolated by the inositol-fess death enrichment procedure have been deposited in the Stock Center. Conidia of strain inl a were treated with sufficient UV for 50-80% kill and then plated in minimal sorbose agar and incubated 3-5 days at 33°C or 37°C. The plates were then transferred to 22°C and supplemented with inositol. Colonies arising subsequently were isolated and tested for temperature sensitivity. Three to five percent of the isolated colonies were temperature sensitive, but half of these were discarded because of poor growth or conidiation even at the permissive temperature\*

No mutant was found which was blocked specifically in Conidial germination. That is, when the mutant conidia were permitted to germinate and start growing in race tubes at 22°C and then shifted to 37°C, growth was arrested in all cases. Most of the mutants stopped quickly (2-4 h) after the temperature shift, though one (165C) continued to grow for many hours before finally stopping.

Results of linkage studies and some other properties of the mutants are summarized in Table 1.

There are five cases in which two or more mutants appear to be allelic. These groups are indicated in Table 1 by the spacing. They include two groups of four mutants each and three groups of two mutants each. Mutants grouped in this manner failed to complement each other in heterokaryon tests for ts+ function. Complementation tests were performed for nearly all pairwise combinations of the 34 mutants. The result was positive in all cases except those indicated here. In four of the groups the Gross tests and the heterokaryon tests were all consistent with the members of a group being allelic. In the group made up of 33, 134C, 16J and 21T, the evidence is doubtful. Heterokaryon tests were incomplete with 16J be-

TABLE 1.

## Properties of temperature-sensitive mutants

Mutant	Linkage Group	Linkage notes	Reparability	Germination	Growth rate 28° C/20° C
6B	I	These all map between <u>mt</u> and <u>his-3</u>	I	2% 1d	0/1.4
145C	I		I	5% 1d	
19D	I		I	10% 1d	0/1.5
47D	I		I	0	0/2.9
60C	I	between <u>mt</u> and <u>his-8</u>	lysine	0	4.3/2.9
120C	IR	between <u>his-3</u> and <u>al-2</u>	I	15% 2d	0/1.6
151C	IL	distal to <u>mt</u>	I	90% 20d	
209C	IL	distal to <u>mt</u>	I	60% 1d	3.4/2.4
6T	IR	distal to <u>al-2</u>	I	2% 1d	0.2/1.4
72c	II	between <u>pvr-4</u>	threonine	5% 1d	0/2.5
38E	II	and <u>arg-5</u>	threonine	10% 1d	1.7/2.3
4M	IIR	very close to <u>um-15</u> (mapped by D. Perkins)	I		1.5/1.7
34C	IVR	proximal to <u>pvr-1</u>	I	0	4.0/2.8
74E	IV?	32% recombination with <u>col-4</u>	I	0	2.9/2.5
26U	IVR	close to <u>col-4</u>	methionine	2% 2d	4.1/3.0
3B	V	all linked to <u>arg-4</u> (but see footnote)	I	20% 2d	2.0/2.6
134c	V		I	15% 1d	
16J	V		I	5% 1d	1.3/2.7
21T	V I		I	10% 1d	2.0/2.7
121C	VR]	distal to <u>inl</u>	I	20% 2d	
181C	VR]		I	40% 2d	0.5/1.5
152C	V?	possible weak linkage to <u>inl</u>	methionine	2% 1d	
165C	VR	distal to <u>inl</u>	I	95% 40d	
58E	V?	36% recombination with <u>inl</u>	I	2% 1d	3.0/2.3
20J	V	7% recombination with <u>inl</u>	I		3.3/2.6
64D	VIR	distal to <u>trp-2</u>	I	90% 15d	0/2.2
61C	VIR]	close to <u>met-7</u>	I	90% 10d	2.4/2.4
62C	VIR]		I	75% 5d	2.4/2.6
23N	?		I	95% 20d	4.0/2.9
74N	?		I	5% 1d	0.2/1.5
29T	?		methionine	50% 1d	
4V	?		threonine	40% 2d	1.3/2.1
105W	?		methionine	0	1.9/2.2
119W	?		methionine	5% 2d	2.0/2.2
<u>inl a</u> (ts+ control)				95% 20d	4.2/3.0

Reparability: I = irreparable (no growth on complete medium at 37°). For the reparable mutants the required growth factor is listed.

Germination: percent germination on solid medium in 7 h at 37°, maximum germ tube length in cell (conidium) diameters.

Growth rate 28°/20°: maximum linear growth rates in mm/h on solid medium in race tubes. This test shows that the various mutants differ as to the upper limit of their permissive temperature range.

cause it became aconidial before the tests were completed. Strain 134C has an unusual growth habit which interferes with heterokaryon formation and resulted in false negatives in some tests. The genetic tests showed all four mutants linked to arg-4 on linkage group V, but some of the tests disagreed as to whether the mutant locus was left or right of arg-4.

We also monitored the following processes in germinating conidia of the various ts mutants at 37°C: macromolecular synthesis (DNA, RNA, protein), rate of increase in mass (dry weight) and nuclear division. Results of these tests can be obtained by writing to me.

The Stock Center strains of all but one of the mutants (16J) are still in the original genetic background. Three of the mutants have been reported in publication. They were placed in the Stock Center earlier and have gene symbols as follows:

34C psi-1 Loo 1975 J. Bacteriol. 121: 286.

4M rip-1 Loo 1975 Neurospora Newsl. 22: 10; Loo et al. 1981 Mol. Cell Biol. 1: 199.

3B ndc-1 Serna and Stadler 1978 J. Bacteriol. 136: 341.

The work on these 34 mutant strains was done in my laboratory over a number of years by myself and several colleagues, notably Melanie Loo, Beverly Kariya, Eva Crane and Leticia Serna. Most of the linkage analysis was done by the late Agnes Towe. - - - Department of Genetics, University of Washington, Seattle, Washington 98195.