THE GENETIC CONTROL OF SPORULATION IN SACCHAROMYCES I. THE ISOLATION OF TEMPERATURE-SENSITIVE SPORULATION-DEFICIENT MUTANTS[1]

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PRESENT knowledge of the biochemical, cytological, and genetic events which accompany meiosis does not provide precise information regarding the nature and number of indispensable functions which must proceed for meiosis to occur. Such information could be obtained if conditional mutants preventing these functions were available for study.

Yeast possesses several properties which make it favorable for the study of meiosis: 1) meiosis proceeds in single cells without the influence of surrounding tissues; 2) it is possible to initiate meiosis at will in synthetic medium by manipulation of cultural conditions; 3) the genetics of this yeast has been extensively studied and there are several well marked linkage groups which may be used to study the effects of meiotic mutations on chromosome segregation and genetic recombination; 4) biochemical techniques applicable to yeast are available and complement the features of yeast as an organism for genetic study.

In yeast, meiosis and ascospore development, collectively termed sporulation, terminate with the production of an ascus containing four ascospores. One would expect that mutants deficient in their ability to sporulate and form asci would represent mutational blocks throughout the process of meiosis and ascospore development. The following report describes the isolation and preliminary characterization of temperature-sensitive mutants of yeast deficient in their ability to sporulate.

MATERIALS AND METHODS

Yeast strain: A homothallic diploid strain of Saccharomyces was employed in this study. This diploid, S41, was obtained from DR. D. C. HAWTHORNE. The genotype of this strain is shown below:

a	*D*	*arg-4*	*acr-1*
α	*D*	*arg-4*	*acr-1*

a, α: mating type alleles
D: diploidization gene (WINGE and ROBERTS 1949)
arg: arginine auxotroph
acr: actidione resistance

Media: The amounts of the various ingredients indicated are those required for the preparation of one liter of medium. *Minimal*-20 g dextrose, 6.7 g Difco yeast nitrogen base, 15 g agar;

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Minimal plus arginine- minimal plus 75 mg arginine; *Glycerol-* 30 ml 96% glycerine, 20 g Bacto-peptone, 10 g yeast extract, 15 g agar; *Sporulation medium-*1 g dextrose, 2.5 g yeast extract, 20 g potassium acetate, 15 g agar; *Yeast Extract Peptone (YEP)*-20 g dextrose, 20 g Bacto-peptone, 10 g yeast extract, 15 g agar.

Ultraviolet irradiation: Approximately 250 cells per plate were irradiated on the surface of agar medium at 25 cm from an 8W General Electric germicidal lamp. Irradiation and subsequent operations were performed in dim daylight.

Sporulation and counting procedure: Diploids to be sporulated were grown for 48 hrs at 30°C on solid YEP medium. The colonies were then replica plated to sporulation medium. After five days of incubation at 20°C, 30°C, or 34°C, the percent asci was determined by haemocytometer counts of a water suspension of a portion of the sporulated replica. For these estimates no attempt was made to distinguish buds from cells, each was counted as a cell. Thus, the percent asci is a minimum estimate of the frequency of meiotic cells since a fraction of the small buds did not contain nuclei. This procedure was adopted because it eliminated subjective decisions regarding the total cell number and allowed rapid scanning of sporulated cultures without resorting to nuclear staining techniques.

Ascus dissection: Sporulated cultures were prepared for dissection by treating the asci with snail digestive juice to soften the ascus wall. The spores of the ascus were then separated by micromanipulation (HAWTHORNE and MORTIMER 1960).

Tests for mating ability: Derivatives of strain S41 to be tested for mating response were crossed to a and α haploid tester strains which required adenine for growth. After 48 hrs of growth on YEP medium at 30°C the crosses were replica plated to minimal medium. Confluent growth of a replica on this medium was scored as a positive mating response.

EXPERIMENTAL RESULTS AND DISCUSSION

The sporulation-deficient mutants were isolated in a homothallic strain of Saccharomyces carrying the *D* (diploidization) gene described by WINGE and ROBERTS (1949). The life cycle of a *D* gene strain is summarized in Figure 1. A homothallic diploid homozygous for the *D* gene produces haploid ascospores which diploidize after a few mitotic divisions (HAWTHORNE 1963). The *D* gene strain was employed since it suggested a convenient method of obtaining mutations affecting sporulation in homozygous condition in diploid cells. One would expect that a mutation affecting sporulation induced in a haploid ascospore carrying the *D* gene would eventually appear in homozygous condition in the diploid cells composing the ascospore colony. By this technique both dominant and recessive mutations may be obtained in diploid cells which may then be tested directly for their ability to sporulate.

Ultraviolet induction of sporulation-deficient mutants: Mutants affecting sporulation were induced in strain S41 by ultraviolet irradiation. Temperature-sensitive mutants were sought to facilitate the genetic analysis of meiotic mutations which would require sporulation and tetrad analysis. A culture of S41 was grown to stationary phase on YEP medium and was replica plated to sporulation medium. At the completion of sporulation the culture, consisting of 78% asci and 22% unsporulated cells, was washed once with distilled water and plated on minimal plus arginine medium. The cells were irradiated with ultraviolet light for 10 sec. This dose resulted in approximately 40% survival. Since 10 sec of ultraviolet light results in 50% killing of diploid cells approximately 73% of the surviving colonies resulted from the growth of a single ascospore.

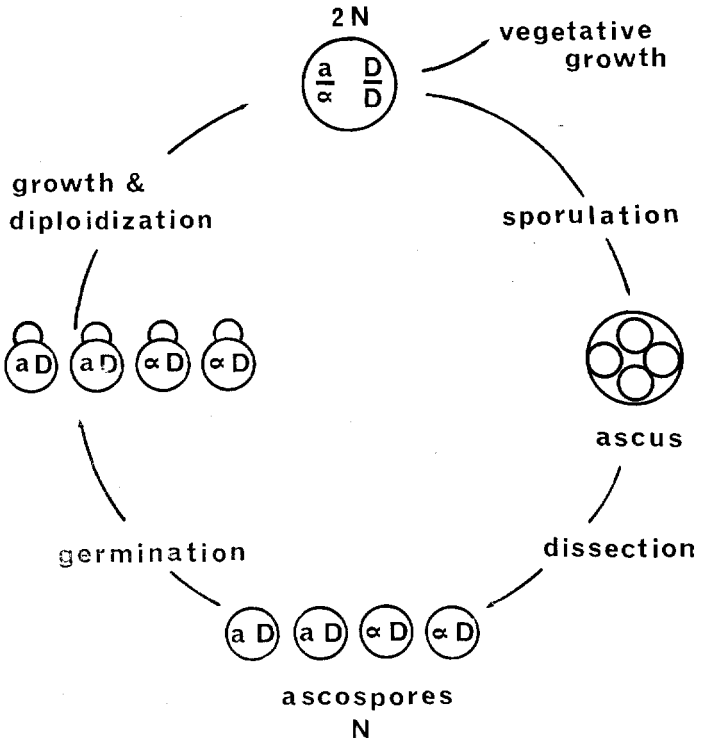

FIGURE 1.—The cell cycle of a *D* gene homothallic yeast strain. Diploidization occurs during the vegetative growth of a spore as a result of copulation of haploid cells following directed mutation of either the *a* or *α* mating type alleles to its opposite allele (HAWTHORNE 1963).

After six days of growth at 30°C, 896 survivors were transferred to YEP plates and were replica plated to minimal, minimal plus arginine, glycerol, and sporulation media. Survivors which did not grow on glycerol or sporulation media were not considered further since they are likely to represent respiratory-deficient petite mutants unable to utilize acetate which is the prime carbon source of the sporulation medium (EPHRUSSI, HOTTINGUER, and CHIMENES 1949). In addition only those survivors which retained their arginine requirement and grew well on the minimal plus arginine media at all three temperatures were examined further.

At five days a portion of each sporulated replica was examined in a water suspension for the presence of asci. Optimum sporulation of the parental strain occurred at 30°C with near optimum sporulation frequencies at 20°C and 34°C (see Table 1). Survivors which exhibited lower sporulation than the wild-type parental strain were tested further. 142 survivors were in this class. Each putative mutant was purified by restreaking on YEP medium and a single colony was tested for its ability to sporulate at the three temperatures. At this second screening the percentage of asci at five days was measured by haemocytometer counts of at least 500 cells per determination.

Among the 142 putative mutants tested 75 were found to differ significantly

TABLE 1

Percent asci at 20°C, 30°C, and 34°C of strain S41

Clone	20°C	30°C	34°C	20°C / 30°C	20°C / 34°C	30°C / 34°C
1	46	78	61	0.6	0.8	1.3
2	60	75	53	0.8	1.1	1.4
3	49	80	50	0.6	1.0	1.6
4	58	78	40	0.7	1.4	2.0
5	52	67	42	0.8	1.2	1.6
6	44	78	44	0.6	1.0	1.8
7	58	68	49	0.9	1.2	1.4
8	60	85	41	0.7	1.5	2.1
9	60	72	41	0.8	1.5	1.8
10	59	69	35	0.9	1.7	2.0
Mean	54.6	75.0	45.6			
Standard Deviation	6.3	5.8	7.6			

from the parental strain. The percent sporulation of 75 strains are summarized in Table 2. The mutants are ordered with respect to their percent sporulation at 20°C. For each mutant the ratios of the percent asci at 20°C, 30°C, and 34°C have been calculated and may be compared with the values exhibited by the parental strain (Table 1). All of the mutants listed in Table 2 were tested at least twice for their percent asci at the three temperatures.

The mutants which exhibited no asci at all three temperatures were tested for their mating ability since those mutants could represent 1) haploids in which the *D* gene function was lost or 2) *aa* or $\alpha\alpha$ diploids arising by mitotic exchange in diploid cells. Both possibilities could be eliminated by a mating test since *a*, α, *aa*, $\alpha\alpha$ strains mate but do not sporulate (ROMAN, PHILLIPS, and SANDS 1955). None of the mutants demonstrated mating ability.

Temperature-sensitivity of the sporulation-deficient mutants: The ratios of the percent asci at 20°C, and 34°C of the 69 mutants listed in Table 2 which produced asci at one or both temperatures are shown as a frequency distribution in Figure 2. The ratios of 4/69 of the mutants fall within the range (0.8–1.7) exhibited by the parental strain (Table 1). The remaining 65 mutants are temperature-sensitive; 16 are sensitive to cold and 49 are sensitive to heat. Thus 65/75 (84%) of the mutants isolated display temperature-sensitivity.

Ascospore survival of sporulation-deficient mutants and their segregants: To pursue the genetic analysis of the mutants it was necessary to determine whether the mutations were in homozygous condition as expected, and to assess their effects on ascospore viability. A sample of 23 mutants was sporulated at the permissive temperature and the asci were dissected. The results with respect to ascospore survival are shown in Table 3. The ascospore viability of seven mutants was similar to wild type and ranged from 86% to 100%. Twelve mutants showed spore survival values between 5% and 50%; the remaining four mutants yielded no surviving ascospores.

TABLE 2

*Percent asci at 20°C, 30°C, and 34°C of sporulation-deficient mutants**

Mutant No.	20°C	30°C	34°C	20°C / 30°C	20°C / 34°C	30°C / 34°C
1	< 0.2	< 0.2	< 0.2	..	..	..
14	< 0.2	< 0.2	< 0.2	..	..	..
17	< 0.2	< 0.2	< 0.2	..	..	..
18	< 0.2	< 0.2	< 0.2	..	..	..
39	< 0.2	< 0.2	< 0.2	..	..	..
66	< 0.2	< 0.2	< 0.2	..	..	..
9	< 0.2	< 0.2	1.3	..	< 0.2	< 0.2
85	< 0.2	< 0.2	9.7	..	< 0.1	< 0.1
13	0.9	12.9	3.1	0.1	0.3	4.2
5	1.0	4.4	4.1	0.2	0.2	1.1
6	1.1	1.0	3.2	1.1	0.3	0.3
93	1.6	< 0.2	< 0.2	> 8.0	> 8.0	..
60	1.6	3.9	< 0.2	0.4	> 8.0	>19.5
86	2.0	< 0.2	0.5	>10.0	4.0	< 0.4
10	2.3	0.9	5.6	2.6	0.4	0.2
2	2.6	23.0	49.1	0.1	0.1	0.5
48	2.8	< 0.2	3.8	>14.0	0.7	< 0.1
30	2.9	59.3	6.2	0.1	0.5	9.6
87	3.6	1.1	< 0.2	3.3	>18.0	> 5.5
28	3.6	20.1	26.8	0.2	0.1	0.8
20	5.9	4.4	0.6	1.3	9.8	7.3
15	6.8	35.9	10.6	0.2	0.6	3.4
19	6.9	4.7	1.0	1.5	6.9	4.7
64	7.9	< 0.2	< 0.2	>39.5	>39.5	..
54	7.9	16.8	3.1	0.5	2.5	5.4
11	8.4	33.7	23.7	0.2	0.4	1.4
12	8.8	33.1	20.7	0.3	0.4	1.6
52	8.6	16.9	1.1	0.5	7.8	15.3
84	10.6	7.5	< 0.2	1.4	>53.0	>37.5
62	11.7	8.8	0.4	1.3	29.3	22.0
88	12.3	5.4	< 0.2	2.3	>61.5	>27.0
36	12.4	< 0.2	21.1	>62.0	0.6	< 0.1
33	12.4	5.0	7.6	2.5	1.6	0.7
80	13.7	28.0	0.7	0.5	19.6	40.0
61	15.7	11.2	2.4	1.4	6.5	4.7
59	15.6	27.2	12.7	0.6	1.2	2.1
41	16.3	35.2	6.7	0.5	2.4	5.2
16	18.9	52.7	25.4	0.4	0.7	2.1
46	19.6	0.8	7.6	24.5	2.6	0.1
76	20.0	38.4	< 0.2	0.5	>100.0	>192.0
67	20.1	23.5	16.2	0.9	1.2	1.5
100	25.8	29.8	2.2	0.9	11.8	13.6
56	27.8	12.9	0.7	2.2	39.7	18.4
7	28.4	22.7	4.2	1.3	6.8	5.4
82	28.8	< 0.2	3.5	>144.0	8.2	< 0.1
89	30.0	7.2	1.2	4.2	2.5	6.0
3	30.6	10.1	3.5	3.0	8.7	2.9
102	31.2	22.9	0.4	1.4	78.0	57.3

TABLE 2—Continued

Mutant No.	20°C	30°C	34°C	20°C 30°C	20°C 34°C	30°C 34°C
91	33.2	6.6	4.0	5.0	8.3	1.7
79	41.3	36.3	2.4	1.1	17.2	15.1
51	41.3	47.2	5.6	0.9	7.4	8.4
40	42.0	26.3	12.8	1.6	3.3	2.1
71	42.1	33.4	6.9	1.3	6.1	4.9
98	43.5	20.0	0.2	2.2	212.5	100.0
73	43.6	57.2	13.0	0.8	3.4	4.4
90	44.5	13.4	8.2	3.3	5.4	1.6
21	45.8	28.2	12.2	1.6	3.8	2.3
92	49.4	29.6	0.8	1.7	61.8	37.0
78	51.0	55.0	0.9	0.9	56.6	61.1
74	51.2	2.0	< 0.2	25.6	>256.0	>10.0
70	53.5	46.2	19.7	1.2	2.7	2.3
81	53.5	62.5	7.7	0.9	6.9	8.1
65	55.7	43.5	4.3	1.3	12.9	10.1
25	56.8	34.7	15.7	1.6	3.6	2.2
68	56.8	71.8	15.7	0.8	3.6	4.6
24	57.2	83.1	4.9	0.7	11.7	17.0
4	57.8	9.4	< 0.2	6.1	>289.0	>47.0
69	58.0	35.8	4.4	1.6	13.2	8.1
97	58.1	38.0	2.6	1.5	22.4	14.6
57	58.7	53.5	9.8	1.1	6.0	5.5
32	66.8	69.0	17.4	1.0	3.8	4.0
34	69.6	75.8	15.1	0.9	4.6	5.0
83	74.6	42.6	2.1	1.8	35.5	20.3
23	75.0	81.2	17.2	0.9	4.4	4.7
37	78.0	69.2	9.2	1.1	8.5	7.5

* Strains listed as mutant exhibit sporulation frequencies at least three standard deviations lower than the mean value of the parental strain (S41) at one or more of the temperatures tested.

The lethality observed may be due to the sporulation mutants themselves or to the segregation of independently induced recessive lethals. Since mosaic colonies have been observed following mutagenesis of haploid yeast (NASIM and CLARKE 1965; HAEFNER 1967), diploid colonies heterozygous for lethal mutations would not be unexpected following mutagenesis of homothallic haploid ascospores. If lethality were due to the segregation of lethals, surviving ascospore segregants should demonstrate near wild-type spore viability upon further sporulation and dissection. However, no improvement in ascospore viability would be expected where spore death resulted from the sporulation mutation.

To determine whether reduced ascospore viability would persist the segregants obtained from the original mutants were sporulated at the permissive temperature and the resultant asci were dissected. The percent ascospore survival of these segregants is also given in Table 3. The ascospore viability of mutants 81, 78, 37, 56, 61, and 71 remained low. In the case of these six mutants decreased ascospore survival may result from the sporulation mutation itself.

Percent sporulation of segregants of sporulation-deficient mutants: The sporu-

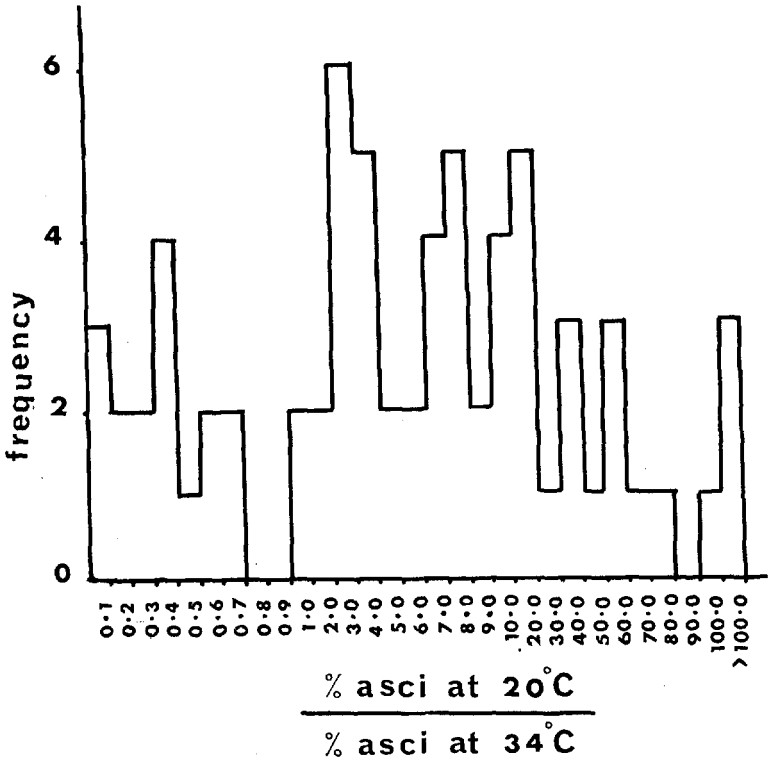

FIGURE 2.—Frequency distribution of the ratios of the percent asci at 20°C/ percent asci at 34°C of 69 sporulation-deficient mutants.

lation frequencies of the segregants of the 19/23 mutants (see Table 3) which yielded viable ascospores are shown in Table 4. In each instance one or two ascospore colonies (eg. 19-1A) were again sporulated at the permissive temperature and dissected to determine the sporulation ability of their segregants (eg. 19-1A-1A, 1B, 1C, 1D).

The mutants may be divided into two broad classes: those which produced segregants whose sporulation frequencies were uniform and similar to the original mutant (e.g. 41, 83, 74, 89) and those which exhibited variable sporulation frequencies through two cycles of vegetative growth, sporulation, and dissection (e.g. 76, 81, 78, 61). In general those mutants which gave high ascospore survival produced segregants whose sporulation frequencies were uniform while those mutants which exhibited intermediate or low ascospore survival yielded segregants with variable sporulation frequencies.

Since ascus production represents the termination of the sporulation process, thus the mutants collected should represent lesions throughout meiosis and ascospore development. Low ascospore survival and variable sporulation frequencies may reflect disturbances of chromosome segregation and assortment occurring at the permissive temperature. The mutants which exhibited high spore survival

TABLE 3

*Ascospore viability of sporulation-deficient mutants and their segregants**

Mutant No.	Percent ascospore survival	Segregants	Percent ascospore survival
19	100	19–1A, 19–1B	83, 83
41	100	41–1A, 41–1B	96, 67
83	100	83–1A, 83–1B	92, 80
74	96	74–1A, 74–1B	92, 79
97	93	97–2A, 97–2B	84, 50
57	88	57–1C, 57–1D	93, 92
89	86	89–1D	100
51	45	51–1C, 51–3B	63, 88
79	41	79–1B	100
76	29	76–2G, 76–3A	92, 83
69	21	69–2A, 69–3A	83,82
65	8	65–1A	96
92	8	92–1A	86
81	50	81–5B	13
78	31	78–1A, 78–6A	21, 15
37	13	37–2A	42
56	8	56–1A	13
61	8	61–2A	50
71	5	71–1A	65
33	0		
82	0		
90	0		
102	0		

* Approximately 28 ascospores of each strain were tested for viability. The ability of an ascospore to form a colony on YEP medium was used as the criterion of viability.

and uniform sporulation frequencies at the permissive temperature could be concerned with functions in the sporulation cycle which precede or follow chromosome segregation.

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SUMMARY

As part of a study of the genetic regulation of meiosis and sporulation in yeast, temperature-sensitive mutants deficient in their ability to sporulate have been isolated in a homothallic strain of yeast carrying the *D* (diploidization) gene. Haploid ascospores obtained by sporulation of a diploid strain homozygous for the *D* gene were irradiated with ultraviolet light. The surviving diploid colonies were examined for their ability to sporulate at 20°C, 30°C, and 34°C. By this

TABLE 4

Percent sporulation of segregants of sporulation-deficient mutants

Mutant No.	Spore	Percent asci 20°C	Percent asci 34°C	Mutant No.	Spore	Percent asci 20°C	Percent asci 34°C	Mutant No.	Spore	Percent asci 20°C	Percent asci 34°C
19	1A	3.9	7.7	74	1A	61.5	0.4	89	1A	4.9	2.8
	B	6.9	12.3		B	66.2	0.2		B	30.8	1.8
	C	6.5	9.2		C	65.9	0.8		C	12.2	0.8
	D	4.3	5.4		D	69.8	0.4		D	29.3	1.7
19–1A	1A	3.1	16.5	74-1A	1A	40.2	<0.2	89-1D	1A	31.0	1.7
	B	8.1	27.6		B	41.7	<0.2		B	25.5	0.6
	C	5.8	19.4		C	38.6	<0.2		C	27.4	3.2
	D	6.4	24.6		D	35.5	<0.2		D	21.7	1.2
19–1B	1A	5.1	17.1	74-1B	1A	42.9	<0.2	51	1A	51.5	30.5
	B	7.5	16.8		B	45.0	<0.2		B	56.5	22.4
	C	4.4	13.5		C	43.6	<0.2		C	15.0	0.4
	D	3.8	13.7		D	45.6	<0.2				
41	1A	56.5	5.9	97	2A	50.8	20.3		3A	9.7	1.9
	B	40.0	4.7		B	48.9	16.2		B	50.2	22.0
	C	39.3	12.5		C	52.8	25.2		C	15.8	7.6
	D	22.0	0.5		D	46.5	18.7				
41–1A	1A	54.9	2.7	97-2A	1A	42.8	1.8	51-1C	3A	9.6	2.0
	B	59.1	1.0		B	49.4	5.6		B	11.2	4.9
	C	56.3	2.1		C	44.3	7.4		C	12.8	4.5
	D	52.4	1.5		D	47.9	4.2		D	14.3	4.5
41–1B	1A	41.8	1.2	97-2B	1A	46.6	8.5	51-3B	1A	43.3	3.8
	B	40.1	1.8		B	42.1	6.8		B	43.4	0.2
	C	45.3	3.9		C	20.0	6.5		C	39.5	1.0
	D	35.5	2.9		D	39.8	8.2		D	45.4	<0.2
83	1A	56.8	1.1	57	1A	52.0	24.4	79	1A	<0.2	<0.2
	B	52.4	1.6		B	60.0	44.5		B	34.5	10.2
	C	60.4	0.4		C	64.2	43.2		2A	0.2	<0.2
	D	58.8	1.0		D	60.1	22.5		B	<0.2	<0.2
83-1A	1A	62.8	0.4	57-1C	1A	50.1	39.2	79-1B	1A	47.4	1.8
	B	61.6	0.4		B	41.4	18.7		B	52.3	3.1
	C	66.9	<0.2		C	37.3	5.2		C	56.3	6.5
	D	68.5	<0.2		D	35.2	11.5		D	55.8	2.5
83-1B	1A	59.6	1.6	57-1D	1A	37.6	20.3				
	B	63.4	1.0		B	32.1	10.6				
	C	61.3	0.5		C	39.6	12.6				
	D	67.7	0.4		D	37.3	11.6				
76	1A	12.2	0.4	92	1A	37.4	4.2	56	1A	25.8	1.4
	2A	20.5	<0.2		2A	3.6	0.2		2A	<0.2	<0.2
	3A	23.2	1.0						3A	<0.2	13.2
	4A	20.6	9.5								

TABLE 4—(Ccontinued)

Mutant No.	Spore	Percent asci 20°C	34°C	Mutant No.	Spore	Percent asci 20°C	34°C	Mutant No.	Spore	Percent asci 20°C	34°C
76-2A	1A	2.0	<0.2	92-1A	1A	37.0	0.6	56-1A	1A	12.9	11.7
	B	12.0	<0.2		B	49.3	1.0				
	C	0.6	<0.2		C	59.0	2.0				
	D	19.3	<0.2		D	53.4	0.8				
76-3A	1A	23.2	6.7	81	1A	10.6	2.6	61	2A	34.0	12.8
	B	24.7	8.6		B	15.7	1.8				
	C	21.1	10.5		5A	8.3	0.4				
	D	14.5	5.2		B	24.9	2.2				
69	1A	26.6	0.2	81-5B	1A	32.8	5.0	61-2A	1A	17.3	4.1
	2A	36.3	0.2		2A	<0.2	<0.2		B	23.9	7.1
	3A	46.7	0.2		B	23.2	1.5		C	27.6	15.1
	4A	35.8	0.2								
69-2A	1A	42.2	2.0	78	1A	55.1	23.3		2A	35.2	8.2
	B	39.0	<0.2		B	<0.2	<0.2		B	39.4	13.3
	C	38.7	0.2		C	72.7	31.9				
	D	34.1	1.3		6A	9.7	1.0				
69-3A	1A	45.3	16.5	78-1A	1A	60.6	55.2	71	1A	21.6	20.0
	B	49.5	11.8		2A	61.8	51.9		2A	<0.2	<0.2
	C	46.1	12.5								
	D	53.5	9.5								
65	1A	51.4	12.7	78-6A	1A	13.4	7.7	71-1A	1A	30.0	15.6
					2A	<0.2	<0.2		B	29.2	18.6
					3A	<0.2	<0.2		C	18.9	14.1
65-1A	1A	59.1	38.3	37	1A	<0.2	<0.2		2A	25.7	16.2
	B	64.5	54.1		2A	46.4	0.4		B	19.3	14.4
	C	72.2	42.2								
	D	70.0	41.7								
				37-2A	1A	50.1	0.2				
					B	52.1	0.2				
					C	59.6	<0.2				
					2B	60.0	<0.2				
					C	24.2	<0.2				

technique 75 mutants deficient in their ability to form asci have been obtained. 65 of the mutants are temperature-sensitive; 16 are sensitive to cold and 49 are sensitive to heat.

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