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Temperature-Sensitive Multicellular Mutants of *Wangiella dermatitidis*

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Three temperature-sensitive morphological mutants of *Wangiella dermatitidis* were isolated and characterized. The mutants grew in the yeastlike morphology at the permissive temperature (25°C) but expressed a multicellular (Mc) phenotype at the restrictive temperature (37°C). Cultures of Mc 2 and 3 incubated at the restrictive temperature showed rapid reductions in the percentage of budded cells in the population. In contrast, budding continued for several generations in cultures of Mc 1. Incubation of cultures of Mc 2 and 3 at the restrictive temperature for 48 h resulted in nearly total conversion of yeastlike cells to the multicellular form; about 50% of the cells of Mc 1 had converted to multicellular forms after 48 h at the restrictive temperature. Studies using radiolabeled compounds documented that DNA, RNA, and protein synthesis continued at the restrictive temperature. The results suggest that multicellularity is the result of inhibition of bud emergence and cell separation without inhibition of growth, nuclear division, and cytokinesis.

The multicellular (Mc) morphology is one of several vegetative phenotypes expressed by the pathogenic fungus *Wangiella dermatitidis* (19). Other morphologies include a budding yeast form and moniliform and true hyphal forms (6, 15). Although the multicellular form is unique and sometimes characteristic of the fungus in infected tissue, the mechanism regulating the transition from the yeastlike to the multicellular form is not understood and has been studied little. The paucity of information relating to the transition resulted from a general inability to induce the multicellular morphology in vitro (1, 10). This difficulty was partially resolved with the observation that acidic cultural conditions induce the yeast-to-multicellular form transition (19).

Under acidic conditions, conversion of yeastlike forms to the multicellular morphology is characterized by cessation of bud formation followed by wall thickening, cellular enlargement, and septum formation at sites remote from the normal position between the mother and daughter cells. Subculture of the multicellular forms under nonacidic conditions promotes hyphal development (19).

Investigations pertaining to the mechanisms regulating the vegetative morphogenesis of *W. dermatitidis* under acidic conditions present many experimental difficulties. For this reason we isolated temperature-sensitive (Ts) mutants which express the multicellular morphology at the restrictive temperature (37°C) but retain the capability of budding growth at the permissive temperature (25°C). These mutants differ from the parental strain which grows equally well at both 25 and 37°C as budding yeastlike cells. In this report we describe three multicellular mutants which are designated Mc 1, 2, and 3. Culture of the mutants at 37°C results in the disappearance of budded cells in the populations, followed by the appearance of multicellular forms. The effects of the mutations are easily reversed by incubating multicellular forms at 25°C. This induces the multicellular forms to produce colonies composed of yeastlike cells. The behavior of the multicellular mutants suggests that discrete cell cycle events may be responsible for the induction of the various phenotypes of this fungus. The most likely yeast cell cycle event affected by the mutations is bud emergence.

MATERIALS AND METHODS

Organism and growth conditions. *W. dermatitidis* 8656 (originally received from C. W. Emmons, National Institutes of Health, Bethesda, Md.) was obtained from B. H. Cooper (Baylor University Medical Center, Dallas, Tex.). This strain was recently evaluated by McGinnis (11, 12), who determined it is accommodated by the new genus *Wangiella* (11, 12). Although there still may be some disagreement as to the proper taxonomic positions of this organism (3), we will refer to strain 8656 as *W. dermatitidis*.

Both wild-type strain 8656 and the mutants derived from it were grown in a broth medium (CDY) containing (in grams per liter): dextrose, 30; $NaNO_3$, 3;

K_2HPO_4, 1; $MgSO_4$, 0.5; $FeSO_4$, 0.01; and yeast extract, 1. The pH of the medium was adjusted to 6.5 prior to the addition of $FeSO_4$ and the yeast extract. Yeast extract, Sabouraud dextrose agar, and Sabouraud dextrose broth were obtained from Difco Laboratories, Detroit, Mich.

The inoculum used in all experiments consisted of yeastlike cells in the exponential phase of growth at an initial concentration of about 10^6 cells/ml. The inoculum was obtained from cultures grown at 25°C in CDY broth and incubated with shaking on a New Brunswick G-10 gyratory shaker rotating at 200 rpm. Experimental cultures incubated at 37°C were shaken at 250 rpm in a New Brunswick Psychrotherm incubator shaker. Incubation of cultures to determine the temperature sensitivity of the mutants was carried out in a New Brunswick G-76 water bath shaker. The CDY medium used in all experiments was prewarmed to the appropriate temperature prior to inoculation.

Mutant isolation. Exponentially growing cells of *W. dermatitidis* were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (0.04 mg/ml, final concentration in Sabouraud dextrose broth) for 4 h; this procedure resulted in a 1 to 2% survival. After the mutagenesis, the cells were washed, suspended in fresh medium (Sabouraud dextrose broth), dispensed into several flasks, and grown for 24 h at room temperature. Samples from these cultures were diluted, plated on Sabouraud dextrose agar, and incubated at 25°C. Approximately 6,000 colonies were replica-plated to a series of duplicate plates. One series was incubated at 25°C and the other was incubated at 37°C. Cells from about 500 colonies which exhibited abnormal morphologies or scant growth at 37°C, but normal colonies at 25°C, were examined microscopically for the presence of multicellular forms. Three temperature-sensitive multicellular mutants were isolated. These mutants have been maintained at the permissive temperature by subculturing both in liquid medium and on agar slants for 2 years without any apparent change in phenotype.

Photomicroscopy. Phase-contrast micrographs were obtained by use of a Zeiss Universal microscope equipped with a 100× oil-immersion objective. Kodak SO 410 film was used for all exposures.

Measurement of cellular parameters. Enumerations of colony-forming units (CFU) were carried out by plating serial dilutions on Sabouraud dextrose agar. Plates were incubated at 25°C. Determination of the number of CFU was facilitated by the fact that multicellular forms or yeastlike cells incubated at 25°C gave rise to yeastlike colonies within 4 to 5 days. The number of multicellular forms and of budded cells per 100 fungal units was determined by counting the number of multicellular forms or budded cells and the total number of fungal units in several 40× microscope fields. In each case the values derived were based on a total count of at least 400 units. Enlarged muriform units with one or more septa were scored as multicellular forms.

Macromolecular synthesis. Macromolecular synthesis during conversion to the multicellular morphology was measured by incorporation of [8-^{14}C]adenine into RNA and DNA and of L-[4,5-^{3}H]leucine into protein. The procedure followed was essentially that described by Hartwell (7). To label precursor pools uniformly, inoculum cells were prelabeled by growth for 12 h in CDY to which [8-^{14}C]adenine (2 μCi/ml) and L-[4,5-^{3}H]leucine (4 μCi/ml) were added. Uniform labeling of the cells was accomplished by growth in CDY containing [8-^{14}C]adenine (5 μCi/ml) and L-[4,5-^{3}H]leucine (12 μCi/ml).

The amount of [^{14}C]adenine incorporated into RNA was determined by precipitating a volume of the culture in an equal volume of cold 10% trichloroacetic acid and collecting the precipitate on Whatman GF/A glass fiber filters. The amount of [^{14}C]adenine incorporated into DNA was determined by adding 1.0 ml of 2 N NaOH to 0.5 ml of culture and incubating at room temperature for 24 h, followed by precipitation with trichloroacetic acid at a final concentration of about 20%. The precipitates were again collected using GF/A filters. Since the amount of radioactivity incorporated into DNA represented about 5% of the total amount incorporated into nucleic acids, the RNA counts were not corrected for the amount of DNA represented in these counts. To determine the amount of [^{3}H]leucine incorporated into protein, culture samples were boiled in 10% trichloroacetic acid for 30 min; the precipitates were then collected on GF/A filters.

In all cases the precipitates were washed with 10 volumes of 5% cold trichloroacetic acid. Filters were dried completely and placed in 10 ml of scintillation cocktail consisting of 4 g of 2, 5-diphenyloxazole per 1 liter of toluene. Radioactivity was measured with a Beckman LS 100C liquid scintillation counter. Variations in the number of cells on the filters were found to have only a slight effect on quenching of the tritium label.

Chemicals. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was obtained from Aldrich Chemical Co., Milwaukee, Wis. [8-^{14}C]adenine (55 mCi/mmol) and L-[4,5-^{3}H]leucine (5 Ci/mmol) were purchased from Schwarz Bio Research, Inc., Orangeburg, N.Y. 2,5-Diphenyloxazole was purchased from Amersham Corp., Arlington Heights, Ill.

RESULTS

Morphological changes at 37°C. The temporal sequence of events which occurred during incubation of the mutant and wild-type strains at 37°C is depicted in Fig. 1. At time zero cultures of the mutants and wild type exhibited the yeastlike morphology which was characteristic of cells cultured at 25°C. After 12 h of incubation at 37°C, most cells of both Mc 2 and Mc 3 had ceased budding and become somewhat larger, whereas cells of Mc 1 continued to grow by bud formation. Multicellular forms began to appear in cultures of Mc 2 and 3 after 24 h at 37°C. At this time insoluble extracellular material began accumulating as the multicellular forms enlarged and apparently shed portions of their cell walls. After 48 h at 37°C, cultures of all three mutants exhibited the multicellular morphology. By this time considerable quantities of the insoluble extracellular material had accumulated

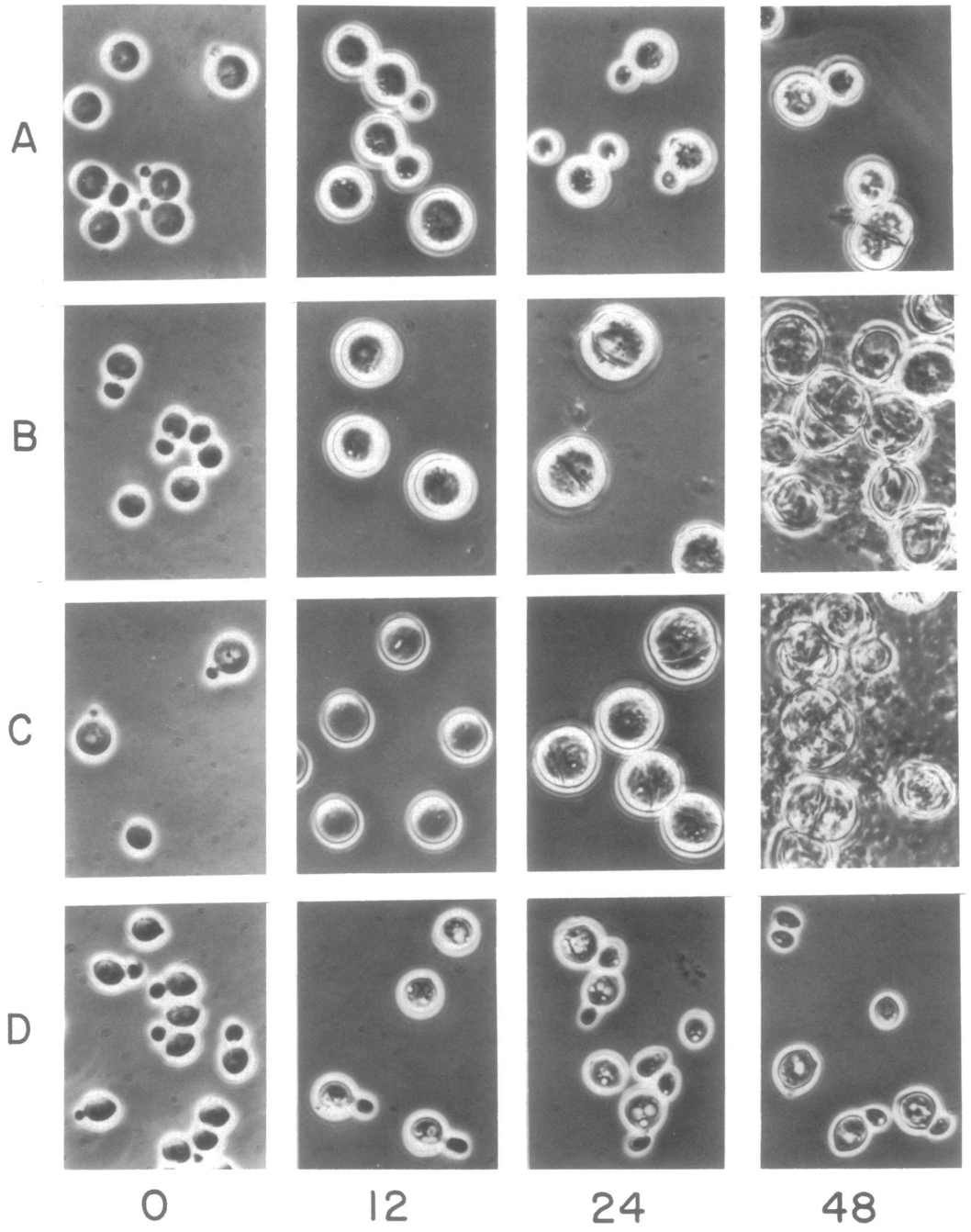

FIG. 1. *Phase-contrast micrographs of the three mutants, Mc 1 (A), Mc 2 (B), and Mc 3 (C), and of the wild type (D) after incubation at 37°C in CDY broth medium. Samples were removed for photography at 0, 12, 24, and 48 h. Multicellular forms were first observed after 12 h at 37°C in cultures of Mc 2 and 3 and after 24 h in the cultures of Mc 1. Magnification, ×1,360.*

in the cultures of Mc 2 and 3. Nearly total conversion of yeastlike cells of Mc 2 and 3 to the multicellular form occurred during incubation at 37°C. In contrast, only about one-half of the population of Mc 1 was multicellular at the end of the incubation period. The wild type exhibited

the typical budding morphology throughout the culture period at the restrictive temperature.

Preliminary mutant characterization at 25 and 37°C. Changes in the percentage of multicellular forms, the percentage of budded cells, and the number of CFU were monitored during the growth of each mutant and wild type at both 25 and 37°C. When grown at 25°C the mutants and wild type behaved similarly with respect to each of these three parameters (see Fig. 2B, 3A, and 4A).

Exposure of Mc 2 and 3 to the restrictive temperature induced initiation of conversion to the multicellular morphology after 12 h (Fig. 2A). Subsequently, these two mutants proceeded to convert almost totally to the Mc phenotype. In contrast, Mc 1 did not initiate conversion until 24 h after exposure to 37°C, and by 48 h only about half of the population was multicellular. No conversion was exhibited either by the wild type at 37°C or by the mutants and wild type at 25°C (Fig. 2).

Incubation of mutant cells at the restrictive temperature significantly affected the percentage of cells with buds in the population (Fig. 3B). Maximum numbers of cells with buds were exhibited in the wild-type culture after 6 h of incubation at 37°C. This maximum was followed by a steady decline which reached almost zero by 48 h. Like the wild type, mutant Mc 1 incubated at the restrictive temperature also attained a maximum in the number of budded cells in the population, although for this mutant the maximum was not exhibited until about 12 h. The number of budded cells of Mc 1 then also decreased steadily to very low levels during the ensuing time. In contrast to Mc 1 and the wild type, Mc 2 and 3 exhibited an immediate reduction in the number of cells with buds. This decrease, which occurred within the first 6 h at 37°C, represented nearly a total elimination of budded cells in the population. At 25°C each mutant behaved like wild type, showing maximum numbers of buds 6 h after inoculation into fresh medium (Fig. 3A).

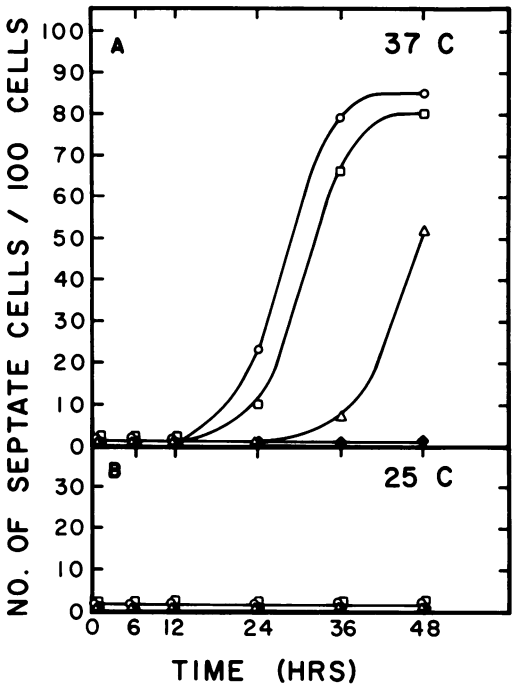

FIG. 2. *Conversion to the multicellular morphology (expressed as the number of septate cells per 100 cells) during incubation of the wild type (◆), Mc 1 (△), Mc 2 (□), and Mc 3 (○) at 37 (A) and 25°C (B). Multicellular forms were never observed in the inocula.*

The effect of temperature on the growth of the mutants as compared to the wild type was equally dramatic (Fig. 4). At the permissive temperature, each of the mutants grew at almost the same rate and to nearly the same final cell concentration as the wild type (Fig. 4A). The growth kinetics of the wild type were modified only slightly by culture at 37°C (Fig. 4B). However, the growth kinetics of each of the mutants cultured at this temperature were greatly modified. At 37°C the growth rate and the final concentration of CFU of Mc 1 were substantially reduced compared to the wild type. The same two parameters were even more reduced for Mc 2 and 3. Both of these mutants displayed minimal increases in CFU during the culture period. Only a slight reduction in viability at 37°C was exhibited by Mc 3 during the entire 48 h of culture, whereas Mc 2 showed reductions in viability after 24 h.

Effect of elevated temperatues. The mutants and wild type were incubated at a number of elevated temperatures to establish the effective temperature range of the mutations. The results at each temperature represent: (i) the maximum percentage of multicellular forms in the mutant and wild-type populations as compared to the percentage of multicellular forms in the 25°C inoculum (Fig. 5); (ii) the maximum percentage of cells with buds (or the minimum percentage when budding was inhibited) in the mutant and wild-type populations compared to the number of budded forms in the 25°C inoculum (Fig. 6); and (iii) the number of CFU after 48 h of incubation (Table 1). The values derived from cultures incubated at 37°C compare favorably with those presented in Fig. 2, 3, and 4, although the results were derived from independent experiments.

Mutant and wild-type populations incubated

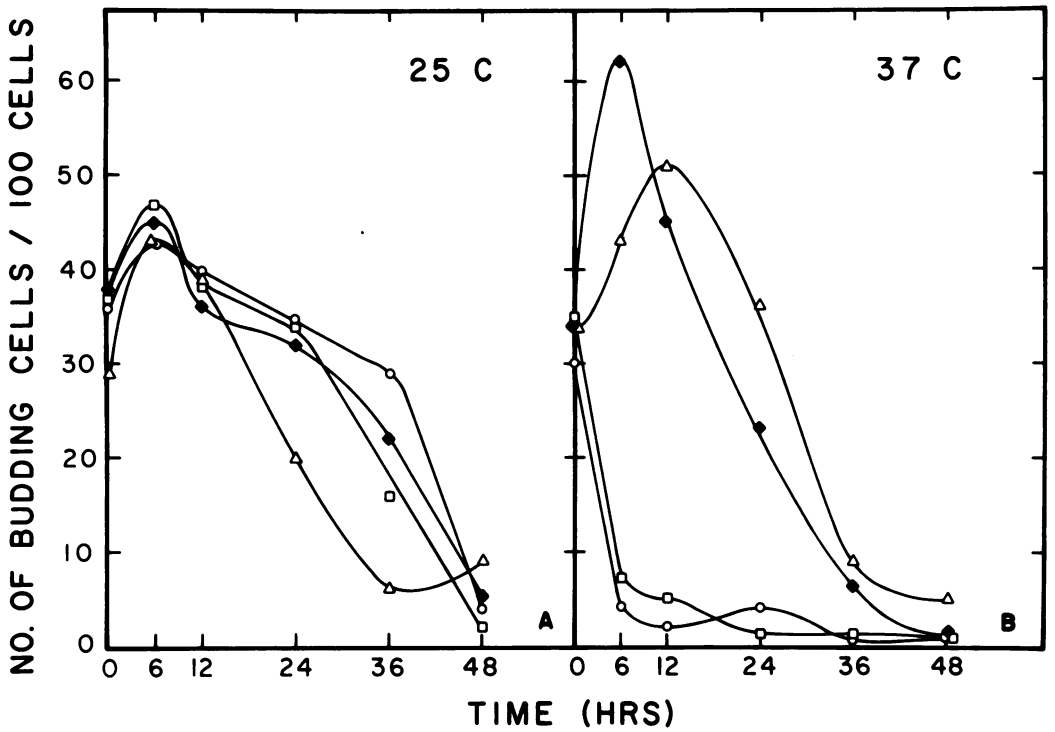

FIG. 3. *Changes in the number of budded cells per 100 cells during incubation of the wild type (◆), Mc 1 (△), Mc 2 (□), Mc 3 (○) at 25 (A) and 37°C (B). Note the very rapid decrease in the number of budded cells of Mc 2 and 3 incubated at 37°C.*

at 35°C generally exhibited the characteristics of cells incubated at the permissive temperature. At this temperature only 10% of the population of Mc 2 and 5% of Mc 3 were observed to be in the multicellular form, and no multicellular forms were observed in the cultures of the wild type and Mc 1 (Fig. 5). Mutants incubated at 35°C exhibited a reduced number of cells with buds compared to that exhibited by wild-type cells (Fig. 6). However, it should be noted that all three mutants exhibited an increase in CFU at 35°C; this is in contrast to the results observed at higher temperatures (Table 1). These results also indicate a slower rate of yeast phase growth for Mc 2 and 3 at this lower temperature.

At both 37 and 39°C almost total conversion of Mc 2 and 3 to the multicellular morphology was observed (Fig. 5). Yeastlike forms of Mc 1 incubated at these temperatures converted only to about the 50% level; the wild type exhibited no tendency toward multicellularity. Budding was greatly suppressed in Mc 2 and 3 as would be expected if cessation of bud formation is a prerequisite for conversion to the multicellular form (Fig. 6). As compared to the wild type, Mc 1 exhibited about a 12% reduction in maximum bud formation at 37°C and a 5% reduction at 39°C. At both 37 and 39°C, Mc 1 exhibited a 5- to 10-fold increase in the numbers of CFU, whereas Mc 2 exhibited lethality at both temperatures (Table 1). Mutant Mc 3 showed about a 25% increase in the number of CFU at 37°C, whereas at 39°C this mutant exhibited lethality.

Temperatures of 42 and 45°C resulted in conversion of only 22% or less of the mutant cells to the multicellular form even though budding was inhibited (Fig. 5 and 6). At these temperatures Mc 2 was unable to exhibit increases in CFU and, in fact, only 40% of the population survived at a temperature of 45°C (Table 1). The lethality expressed by Mc 2 at 37 and 39°C was not as pronounced at 42 and 45°C. An incubation temperature of 42°C reduced the ability of the wild type to grow, and at 45°C lethality was also exhibited by the wild type (Table 1). The wild type incubated at these temperatures arrested growth as budded cells.

Macromolecular synthesis. Incorporation of [^{3}H]leucine into protein and [^{14}C]adenine into DNA and RNA was determined during incubation of the mutants and the wild type at both the restrictive and permissive temperatures. Changes in the numbers of CFU per milliliter were also determined. From these data the

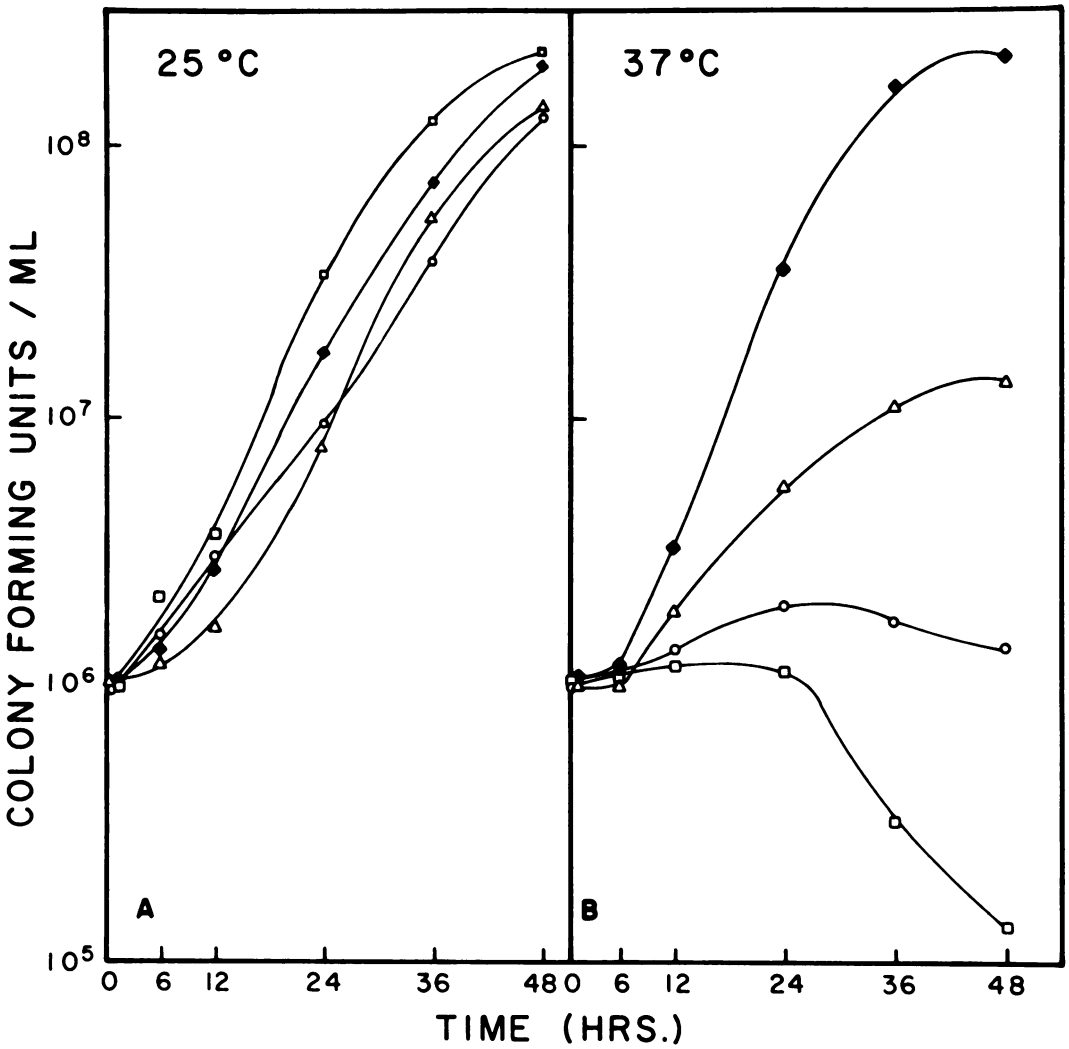

FIG. 4. *Changes in the number of CFU per milliliter during incubation of the wild type (◆), Mc 1 (△), Mc 2 (□), and Mc 3 (○) at 25 (A) and 37°C (B). Numbers of CFU per milliliter were normalized so that the initial value for each culture was 10^6 cells per ml. These data represent the average counts in CFU per milliliter from at least five separate experiments. Note the loss in viability exhibited by Mc 2 after 12 h at 37°C.*

amount of incorporated radiolabeled precursor per CFU was calculated; the results are presented in Fig. 7 as the change in the number of counts per minute per CFU as a function of time.

During incubation at the permissive temperature, the incorporation of [^{3}H]leucine per CFU was fairly constant for all strains with only a slight increase in the amount of radiolabeled precursor being incorporated near the end of the incubation period (Fig. 7A). The wild type incubated at 37°C exhibited similar kinetics of incorporation (Fig. 7B). The amount of [^{3}H]leucine incorporated per CFU of each mutant was substantially higher at 37 (Fig. 7B) than at 25°C. Accumulation of [^{3}H]leucine in protein by Mc 1 and 2 continued steadily during the entire incubation period at 37°C, and by the end of the experiment cellular units of Mc 1 and 2 contained about 13 times as much [^{3}H]leucine as did cells of the wild type. The continued incorporation by Mc 2 is probably only apparent since the experimental procedure may detect protein in units incapable of producing a colony. In comparison to Mc 1 and 2, Mc 3 exhibited somewhat different kinetics of incorporation. The initial rate was comparable to that exhibited by Mc 1 and 2, but accumulation of the radiolabel

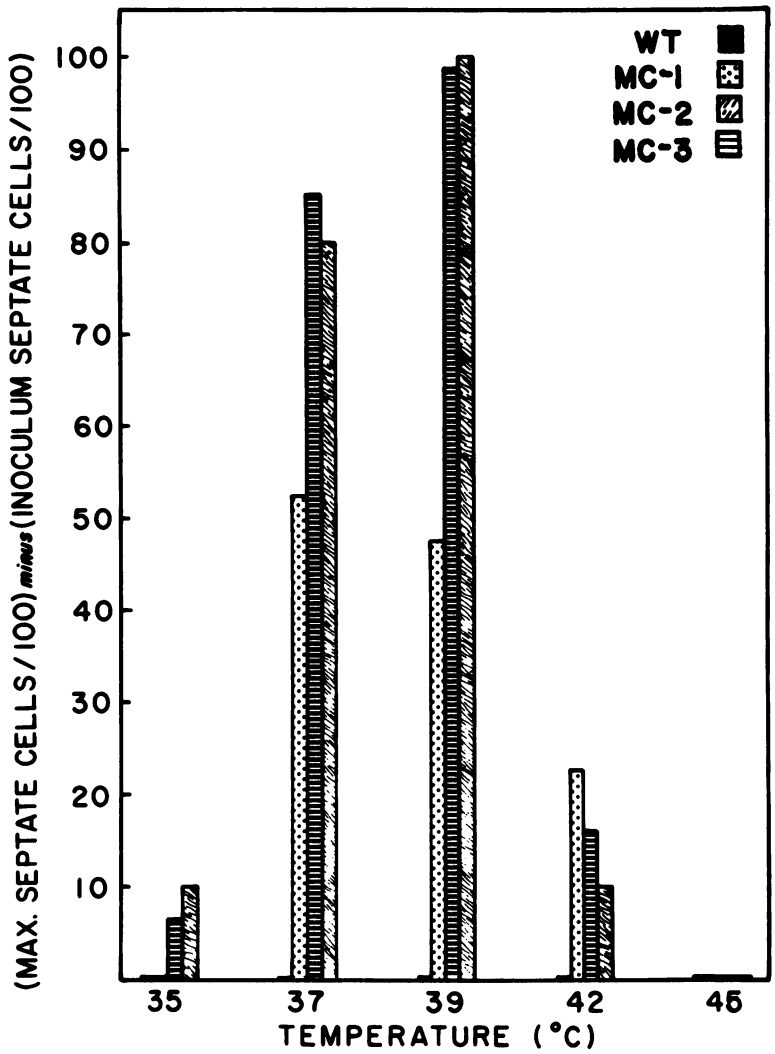

FIG. 5. *Effect of temperature on the maximum percentage of multicellular forms (septate cells) present in the population for cultures of the wild type and the Mc mutants.*

in protein ceased after 24 h at 37°C. In spite of this arrest, Mc 3 ultimately contained three times the amount of incorporated [^{3}H]leucine per CFU as did the wild type.

Determination of the incorporation of [^{14}C]-adenine into RNA in cultures incubated at 25°C produced a pattern similar to that presented for accumulation of [^{3}H]leucine into protein at the same temperature (Fig. 7C). A somewhat constant level of [^{14}C]adenine incorporation per CFU of each mutant and wild type was observed during the culture period. Once again differences were observed at 37°C between the accumulation of radiolabel in the mutants as compared to the wild type (Fig. 7D). Exposure to the restrictive temperature initially produced an increase in the amount of [^{14}C]adenine incorporated for all cultures. However, this rise was only transient for the wild type; the amount of [^{14}C]adenine per CFU eventually decreased to near the original level. The increase in [^{14}C]adenine per CFU was not transient in cultures of the mutants. Incubation of Mc 1 at 37°C resulted in the accumulation of [^{14}C]adenine as RNA for 12 h, after which time the level of [^{14}C]adenine remained fairly constant. At the end of the incubation period, CFU of Mc 1 had incorporated about five times as much [^{14}C]adenine as cells of the wild type. Incorporation of [^{14}C]adenine continued for 24 h in the culture of Mc 3; however,

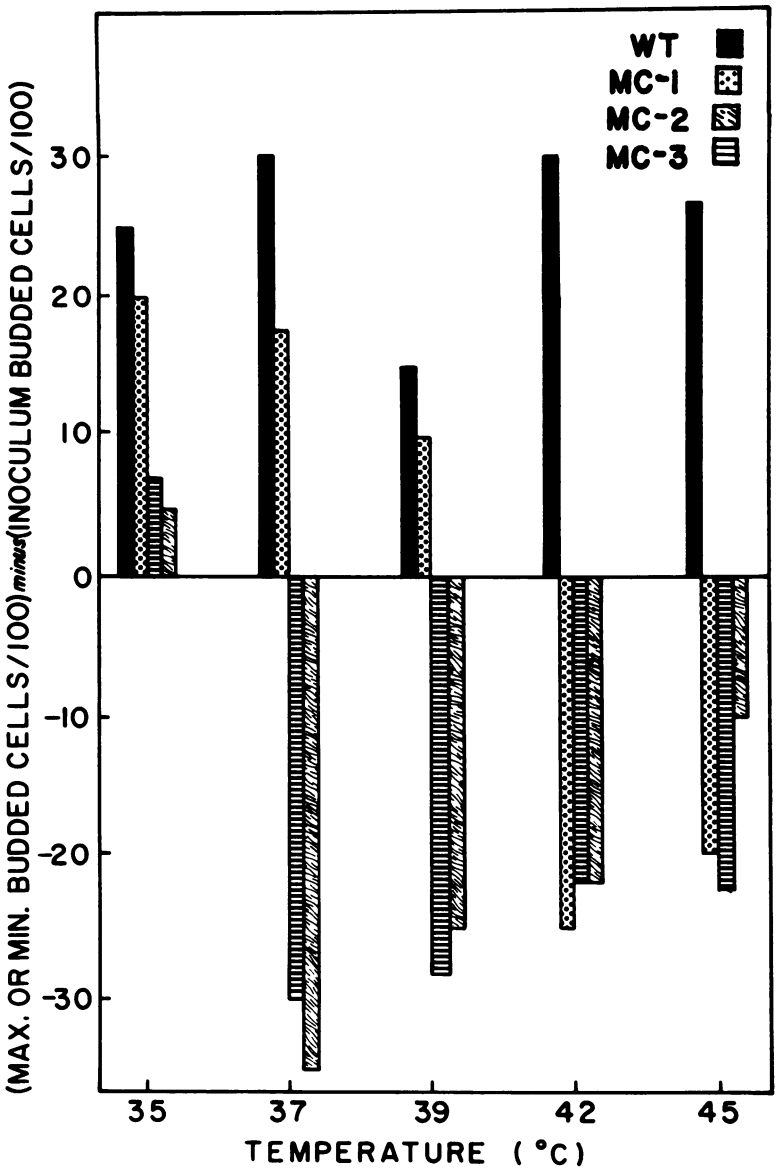

FIG. 6. *Effect of temperature on the maximum (or minimum) percentage of budded cells above (or below) the initial percentage in the inoculum for cultures of the wild type and the Mc mutants. If during incubation only a decrease in the number of budded cells was observed, then the difference between the minimum percentage and the inoculum percentage was plotted; these values are presented as negative values.*

accumulation of radiolabel in RNA stopped at this time, and a decrease in the amount of radiolabel was observed. Unlike Mc 1 and 3, which exhibited an eventual halt in incorporation, Mc 2 appeared to continue to incorporate [^{14}C]adenine as RNA. However, this continuation of incorporation probably only reflects the detection of [^{14}C]adenine in RNA of nonviable as well as viable units of Mc 2. This would result in an unusually high value in counts per minute per CFU.

At 25°C, incorporation of [^{14}C]adenine into DNA proceeded at a nearly constant level for all strains (Fig. 7E). The results presented in Fig. 7F indicate that, at the restrictive temperature, [^{14}C]adenine incorporated as DNA per CFU was significantly higher for Mc 2 and 3 and slightly higher for Mc 1 compared to the wild type. The

TABLE 1. *Final concentration (CFU/ml) of wild-type and mutant strains cultured at several elevated temperatures*[a]

Strain	CFU/ml at:				
	35°C	37°C	39°C	42°C	45°C
Mc 1	1.26×10^8	1.31×10^7	4.68×10^6	5.75×10^5	3.98×10^5
Mc 2	1.62×10^7	1.26×10^5	2.63×10^4	9.12×10^5	6.67×10^5
Mc 3	4.17×10^7	1.26×10^6	3.16×10^5	2.82×10^5	4.47×10^5
WT[b]	1.62×10^8	2.00×10^8	6.76×10^7	7.94×10^6	6.92×10^5

[a] Values above 10^6 represent an increase in CFU per milliliter over the number in the inoculum, whereas values below 10^6 reflect lethality. Cultures were incubated for 48 h.

[b] WT, Wild type.

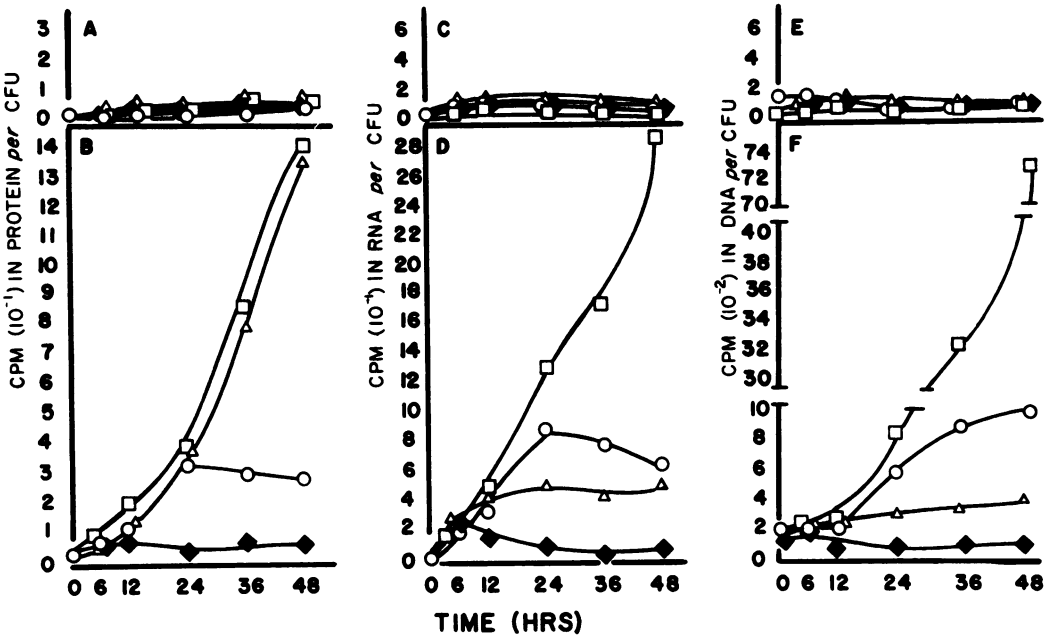

FIG. 7. *Kinetics of incorporation of radiolabeled precursors into macromolecules per CFU. Incorporation of L-[4,5-^{3}H]leucine into hot trichloroacetic acid-insoluble material (protein) was determined for cultures incubated at 25 (A) and 37°C (B). Incorporation of [8-^{14}C]adenine into cold acid-insoluble material (RNA) (C, D) and into alkali-insoluble, cold acid-insoluble material (DNA) (E, F) was determined at 25 (C, E) and 37°C (D, F). Symbols: wild type (◆), Mc 1 (△), Mc 2 (□), and Mc 3 (○).*

increase was observed to occur sometime after 12 h of incubation at 37°C. After 48 h at this temperature, Mc 1 had incorporated about twice the amount of [^{14}C]adenine in DNA as the wild type, whereas Mc 3 had accumulated about 10 times the wild-type value. As with incorporation of radiolabel into protein and RNA, the apparent amount of radiolabel incorporated per CFU of Mc 2 was quite high, the value after 48 h being more than 70 times that of the wild type.

DISCUSSION

The temperature-sensitive multicellular mutants of *W. dermatitidis* represent a new and unique class of fungal morphological mutants. At the permissive temperature these mutants and the parental strain are morphologically indistinguishable and exhibit identical kinetics of growth and synthesis of macromolecules. In addition, nutritional and nuclear staining studies which are in progress indicate that the mutants and wild type when grown at 25°C are not detectably different. The only apparent variance between the mutants and the parental strain is the temperature-dependent induction of the Mc phenotype, one of the three alternative vegetative morphologies characteristic of the parental strain.

Although numerous vegetative morphological

mutants have been isolated in the fungi, the majority express aberrant vegetative phenotypes or arrested vegetative development. Most notable of the former group are the mutant forms of *Neurospora crassa* such as slime (5) and clock (18) and the variants of *Schizophyllum commune* which exhibit altered clamp cell formation (16). Recently, a morphological mutant of the dimorphic fungus *Paracoccidioides brasiliensis* was also isolated (17). This mutant exhibits an aberrant yeastlike cellular phenotype and an altered mycelial colony morphology. Included in the latter group of mutants are the widely studied Ts cdc mutants of *Saccharomyces cerevisiae* (8). Unlike the Ts Mc mutants which express an alternate vegetative morphology at the restrictive temperature, the cdc mutants exhibit arrest of development of their sole vegetative phenotype. The multicellular mutants are also different from the cdc mutants because the effect of the multicellular mutation is reversible, i.e., most multicellular forms of the mutants shifted to 25°C after 48 h at 37°C are able to give rise to yeastlike colonies. Temperature-sensitive mutants of *Aspergillus nidulans* which show vegetative developmental arrest have also been isolated (2, 13, 14). Some of these mutants are defective in chitin synthesis and others, like the cdc mutants, are defective in DNA synthesis, nuclear division, or septation.

Each Mc mutant exhibits multicellularity when subjected to the restrictive temperature, but the expression of this phenotype is somewhat different for each. A rapid cessation of bud formation and nearly complete conversion to the Mc phenotype within 48 h is exhibited by Mc 2 and 3, whereas Mc 1 expresses the budding morphology for several generations before exhibiting the multicellular form. Also, a pronounced degree of lethality at 37°C is expressed by Mc 2, whereas Mc 3 retains viability at this temperature. These results indicate that the mutations carried by the three mutants are either located in different genes or that the three mutations represent different allelic forms of the same gene. It is equally possible that the expression of the Mc phenotype is due to a single gene mutation, the expression of which is modified by Ts mutations elsewhere in the genome.

Rapid arrest of bud formation is characteristic of Mc 2 and 3, whereas Mc 1 only slowly ceases budding growth. Thus, Mc 2 and 3 are similar to first-cycle arrest mutants of *S. cerevisiae* (9). These mutants are able to complete only one or two cell cycles at the restrictive temperature and are probably thermolabile for the function of a gene product. Accordingly, the mutations carried by Mc 2 and 3 may result in the production of a Ts protein which would rapidly bring about the arrest of budding growth. Mc 1 resembles cdc mutants of *S. cerevisiae* which are able to complete several cell cycles after a shift to the restrictive temperature. These mutants are probably thermolabile for a process involved in gene product synthesis. Thus, Mc 1, unlike Mc 2 and 3, would be able to continue bud formation at the restrictive temperature until preexisting protein is degraded or diluted to ineffectual levels.

Near-optimal expression of multicellularity, minimal growth in the yeast phase, and maximal retention of viability of cellular units of Mc 2 and 3 are exhibited at 37°C. Because of these results, 37°C was chosen as the restrictive incubation temperature. An incubation temperature of 39°C produces the maximum conversion to the multicellular form in these two mutants, but it also results in the expression of lethality by Mc 3 as well as by Mc 2. Culture at 35°C does not promote multicellularity; instead, yeast-phase growth persists. Temperatures of 42 and 45°C also do not promote conversion, and at these temperatures a large percentage of wild-type cells accumulate as large budded cells. The results indicate that a temperature of 45°C is probably close to the maximum temperature tolerance for the wild type. The low percentage of Mc forms in mutant populations and the high percentage of budded cells in wild-type populations at 42 and 45°C may indicate that processes leading to further development, including septation, wall synthesis, and other cell cycle events, are inhibited.

The incorporation of radiolabeled precursors into macromolecules at the restrictive temperature tends to reflect the manner in which the Mc phenotype is expressed by each mutant. Incorporation of radiolabeled precursors into multicellular forms of Mc 2 and 3, which is significantly higher than for cells of the wild type, reflects the rapid cessation of budding growth, the enlargement of the cellular unit, and the increase in the number of cells in the multicellular forms. Since accumulation of incorporated precursors is much higher for Mc 2 than for Mc 3, apparent incorporation into the multicellular forms of Mc 2 is probably also due to the detection of radiolabel in nonviable units. In agreement with the late cessation of budding growth and delayed expression of the multicellular morphology of Mc 1, this mutant incorporates amounts of precursor as RNA and DNA that are somewhat higher than the wild type. However, the amount of precursor incorporated as protein is unexpectedly high for Mc 1, suggesting that protein degradation may be defective, pos-

sibly due to the lack of protease activity. It is also possible that, following a shift to the restrictive temperature, new classes of stable proteins are synthesized. In fact, the existence of a stable enzyme involved in cell wall synthesis has been demonstrated in *S. cerevisiae* (4).

Induction of the multicellular morphology is characterized by the enlargement of the cells destined to become multicellular, the continuation of macromolecular synthesis leading to the accumulation of protein, RNA, and DNA, and the division of the cellular unit. The sequence of events necessary for the continuation of the yeast cell cycle appears to be arrested at some point after the initiation of the cell cycle. At the restrictive temperature the only yeast phase cell cycle events which do not appear to be executed are bud formation and cell separation. The Ts Mc mutants of *W. dermatitidis* are similar to the Ts cdc mutant (cdc 24) of *S. cerevisiae* which is arrested in bud formation (9). Arrest of bud emergence in this mutant results in limited continuation and completion of the nuclear division cycle. However, the multicellular mutants are demonstrably different from cdc 24 in that they are also able to complete cytokinesis in the absence of bud emergence. The ability of the multicellular mutants to complete a normal cell cycle when one event in the yeast cycle is arrested most likely relates to the fact that *W. dermatitidis* is vegetatively polymorphic. *S. cerevisiae* has only one morphological sequence for the completion of the mitotic cell cycle, but *W. dermatitidis* possesses several alternatives for the completion of the cycle. At the restrictive temperature, the Ts lesion carried by the multicellular mutants most likely does not allow for the completion of the cell cycle normally operative during budding growth. Instead, an alternate sequence of events is initiated which leads to the expression of the Mc phenotype.

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LITERATURE CITED

1. **Al-Doory, Y.** 1972. Chromomycosis, p. 118–146. Mountain Press Publishing Co., Missoula, Mont.
2. **Cohen, J., D. Katz, and R. F. Rosenberger.** 1969. Temperature-sensitive mutant of *Asperigillus nidulans* lacking amino-sugars in its cell wall. Nature (London) **224**:713–715.
3. **DeHoog, G. S.** 1977. *Rhinocladiella* and allied genera, p. 118. *In* G. S. DeHoog and E. J. Hermanides-Nijhof (ed.), Studies in mycology, vol. 15: The black yeasts and allied hyphomycetes. CBS, Baarn, The Netherlands.
4. **Elorza, M. V., C. M. Lostrau, J. R. Villanueva, and R. Sentandreu.** 1976. Cell wall synthesis regulation in *Saccharomyces cerevisiae*. Effect of RNA and protein inhibition. Biochem. Biophys. Acta **454**:263–272.
5. **Emerson, S.** 1963. Slime, a plasmodial variant of *Neurospora crassa*. Genetica **34**:162–182.
6. **Grove, S. N., K. B. Oujezdsky, and P. J. Szaniszlo.** 1973. Budding in the dimorphic fungus *Phialophora dermatitidis*. J. Bacteriol. **115**:323–329.
7. **Hartwell, L. H.** 1967. Macromolecular synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. **93**: 1662–1670.
8. **Hartwell, L. H., J. Culotti, J. R. Pringle, and B. J. Reid.** 1974. Genetic control of the cell division cycle in yeast. Science **183**:46–51.
9. **Hartwell, L. H., R. K. Mortimer, J. Culotti, and M. Culotti.** 1973. Genetic control of the cell division cycle in yeast. V. Genetic analysis of cdc mutants. Genetics **74**:267–286.
10. **Jotisankasa, V., H. S. Nielson, and N. F. Conant.** 1970. *Phialophora dermatitidis*; its morphology and biology. Sabouraudia **8**:98–107.
11. **McGinnis, M. R.** 1977. *Wangiella*, a new genus to accommodate *Hormiscium dermatitidis*. Mycotaxon **V**: 353–363.
12. **McGinnis, M. R.** 1977. *Wangiella dermatitidis*, a correction. Mycotaxon **VI**:367–369.
13. **Morris, M. R.** 1976. Mitotic mutants of *Aspergillus nidulans*. Genet. Res. **26**:237–254.
14. **Orr, E., and R. F. Rosenberger.** 1976. Initial characterization of *Aspergillus nidulans* mutants blocked in the nuclear replication cycle. J. Bacteriol. **126**:895–902.
15. **Oujezdsky, K. B., S. N. Grove, and P. J. Szaniszlo.** 1973. Morphological and structural changes during the yeast-to-mold conversion of *Phialophora dermatitidis*. J. Bacteriol. **113**:468–477.
16. **Raper, J. R., D. H. Boyd, and C. A. Raper.** 1965. Primary and secondary mutations at the incompatibility loci in *Schizophyllum*. Proc. Natl. Acad. Sci. U.S.A. **53**:1324–1332.
17. **San-Blas, F., G. San-Blas, and L. J. Cova.** 1976. A morphological mutant of *Paracoccidioides brasiliensis* strain IVIC Pb 9. Isolation and wall characterization. J. Gen. Microbiol. **93**:209–218.
18. **Sussman, A. S., R. J. Lowry, and T. Durkee.** 1964. Morphology and genetics of a periodic colonial mutant of *Neurospora crassa*. Am. J. Bot. **51**:243–252.
19. **Szaniszlo, P. J., P. H. Hsieh, and J. D. Marlowe.** 1976. Induction and ultrastructure of the multicellular (sclerotic) morphology in *Phialophora dermatitidis*. Mycologia **68**:117–130.