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Selection of improved microconidial strains of N. crassa

K. D. Munkres
The University of Wisconsin

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Abstract

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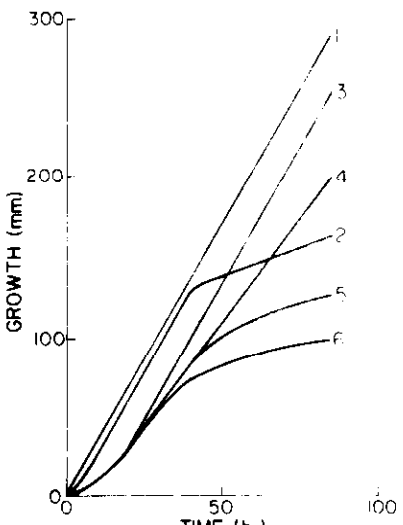
We have investigated a microconidial strain, peach, fluffy, of N. crassa as a possible model for cellular ageing. With the strain pe,fl Y8743m,L A (FGSC #569), we confirmed that young microconidia are low in viability (Barratt 1964 Neurospora Newsl. 6:6; Bayliss and De Busk 1967 Neurospora Newsl. 7:7) and that they die rapidly upon incubation in medium without carbon source. Another defect of this strain is temperature-sensitivity (Fig., curve 6). With mycelial inoculum grown at 30°, growth proceeds for about a day at 37° at a sub-normal rate and subsequently stops. This phenotype segregated in a cross to wild type yielding pe fl isolates which, except for an abnormal lag, grew at wild-type rate without stopping (Fig., curve 3). The segregation for temperature-sensitivity among progeny carrying the morphological markers is interpreted in terms of two genes, temperature-sensitivity-1 and temperature-sensitivity-2, linked to one another (18% recombination), but not to the pe fl genes in group II. The observed 32% recombination of pe fl was in good agreement with Barratt and Garnjobst's (1949 Genetics 34:351) estimate of 31.5%. Temperature-sensitivity-1 alone is expressed at 30° and above,

Patterns of extensional growth of parents and F-1 progeny from a cross of wild-type x peach-fluffy.



Growth on Vogel's minimal in race tubes at 37°.

1) wild-type (74OR-23a) or pe⁺;fl;temperature-sensitive-1⁻;temperature-sensitive-2⁻ (7);
2) pe⁺;fl;temperature-sensitive-1⁺;temperature-sensitive-2 (2);
3) pe;fl;temperature-sensitive-1⁺;temperature-sensitive-2⁻ (4);
4) pe;fl;temperature-sensitive-1;temperature-sensitive-2⁻ (3);
5) pe⁺;fl;temperature-sensitive-1;temperature-sensitive-2 (2) or pe;fl⁺;temperature-sensitive-1;temperature-sensitive-2 (2);
6) pe;fl;temperature-sensitive-1;temperature-sensitive-2 (7) and peach-fluffy (FGSC #569). Parenthetical numbers after each genotype represent the number observed among a total of 27 isolates with the morphological markers.

with an abnormally low steady-state growth rate (Fig., curve 4). This phenotype was not reparable by complete medium; therefore, it appears to be another mutant of the category unknown (Inoue and Ishikawa 1975 J. Gen. Appl. Microbiol. 21:389). The phenotype of temperature-sensitivity-2 alone was expressed at 37° (Fig., curve 2), but not at 30°, and was completely reparable by complete medium when alone or with temperature-sensitivity-1.

The plating efficiency of 5 day-old microconidia on Vogel's minimal sucrose-sorbose at 30° of two of the four pe fl isolates which were free of

the temperature-sensitivity genes was 85-95% in contrast to 1-2% of the original parent. However, these isolates were still not free of undesirable genetic defects. These two isolates were crossed to wild-type. Among 400 random isolates, 80 pe fl were found. Plates of Vogel's sucrose were inoculated with a spot of conidia of each of these 80 and incubated at 37°. Four of the 80 germinated and grew at wild-type rate; the others exhibited a lag in germination. Thus there may be one or more genes, perhaps linked to pe fl, which inhibit the rate of germination of microconidia.

We conclude that the low plating efficiency of microconidia of the original strain is not a phenotype of the pe fl genes; but, rather is probably due to either the temperature-sensitivity-1 gene or another gene closely linked to it. Other mutants of this type, of the category unknown, were described by Inoue and Ishikawa (1975 J. Gen. Appl. Microbiol. 21:389; 1975 Arch Microbiol. 104:1-6), some of which are defective in cell wall or membrane and die from unbalanced growth at the non-permissive temperature.

Selected F_2 lines of pe fl will be deposited with the Fungal Genetics Stock Center. (This work was supported by the College of Agriculture and Life Science and a grant from the National Institutes of Health (GM 21205). The excellent technical assistance of Ms. P. Riese is acknowledged. Contribution No. 2123 from the laboratory of Genetics). - - - Laboratories of Molecular Biology and Genetics, The University of Wisconsin, Madison, WI 53706.