Some mutations affecting perithecial and spore pigmentation

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Some mutations affecting perithecial and spore pigmentation

Abstract
Mutations affecting perithecial and spore pigmentation

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Crude extracts for electrophoretic analysis were prepared as follows: Strains were grown with shaking at 25°C in Vogel's minimal medium. The resulting mycelial growth was harvested on cheesecloth and ground with sea sand and 0.1 M phosphate buffer, pH 7.0, in a pre-cooled mortar and pestle. The resulting slurry was centrifuged at 16,000 r.p.m. (35,000 x g) in a Sorval RC-2, SS-34 rotor. The supernatant was retained and stored frozen until required.

The method employed for the separation of proteins on polyacrylamide disc gels was essentially that described by Barber et al. (1969 Dev. Biol. 20: 105). The 7 cm separating gel contained 7% acrylamide and 0.184% bisacrylamide, and the 0.5 cm stacking gel contained 2.5% acrylamide and 0.625% bisacrylamide. Both electrode chambers contained 0.025 M Tris, 0.19 M glycine buffer, pH 8.3. Approximately 700 µg of protein was applied to each gel, a current of 1 ma/gel was applied and electrophoresis carried out at 5°C until the tracking dye (bromphenol blue) was 0.5 cm from the bottom of the gels. The gels were removed from the tuber and stained with Coomassie Brilliant Blue (Sigma).

Figure 1 is a diagrammatic representation of the acidic proteins visualized following acrylamide gel disc electrophoresis of crude extracts prepared from wild type (74A) and the four mutant strains described. The Rf values for the protein bands of these gels are listed in Table 1. Gels from replicate experiments were not significantly different. Comparisons of the gels indicated that for each rhythmic mutant strain several protein band differences from the wild-type pattern were apparent. This might be expected since Barber, Srb and Steward (ibid.) demonstrated that morphologically different strains of N. crassa exhibited altered electrophoretic protein banding patterns from wild type.

Furthermore, these authors showed that a specific banding pattern was characteristic of a particular morphology, and slight variations in morphology were accompanied by minor changes in protein banding pattern in acrylamide gels. Thus, since the morphologies of the rhythmic mutants examined in this study were distinct from that of wild type, their protein banding patterns were expected to be altered from that of wild type. Also, since the rhythmic mutants were themselves characterized by distinct morphological phenotypes, we hypothesized that their respective protein bands would differ. Indeed, each rhythmic mutant strain showed a number of protein band differences from wild type, these differences being unique for each strain.

In addition, we hypothesized that a common mechanism (oscillating system) might be responsible for rhythmic mycelial growth of Neurospora. If this were so, one might expect that rhythmically growing mutant strains might exhibit certain similarities in their protein compositions that are nevertheless different from wild type. Examination of the gels diagrammed in Fig. 1 indicates that none of the protein differences from wild type were coincident for all four mutant strains. One band (Rf 52) present in wild type was absent from three of the strains, namely 22-214, 24-013 and clock (although not unambiguously so in the latter), but the band was clearly present in the Pk-1 gel. Therefore, these results would tend to argue against the proposed hypothesis under these particular experimental conditions. Nevertheless, the results as a whole present further evidence for pleiotropic protein differences in morphological mutant strains of N. crassa.

Johnson, T. E. Some mutations affecting perithecial and spore pigmentation.

We have isolated seven mutants which fail to make normal, block pigmented perithecia and instead make light yellow perithecia. Perithecial development proceeds normally except for the lack of pigment. Fertile ascospores are produced when either wild type or a mutant is used as the male parent. The genotypically mutant ascospores are unpigmented; they germinate without heat shock a few days after they are shot. A heat shock at this time kills the mutant ascospores.

These mutations may be allelic with the per-I mutation of H. Branch Howe, which maps proximal to 11.x(26201) on the right arm of linkage group V; however, the tests of allelism are not entirely unambiguous at this point. per-I causes the production of perithecia which are light yellow in all their tissues except for the ostiole and the spores. The isolation numbers of our mutants are: per(PBE1), per(PBJ1), per(AB1), per(PB), per(PBT4), per(PBT5), and per(ABT8).
These mutants could be of general use in two different ways: 1) as male parents in test crosses to putative female steriles or in any cross where the identity of the female parent is important; 2) as genetically marked helper nuclei used to correct the female sterile defect in female sterile mutants. We have tested these mutants in both capacities.

In testing putative female sterile strains, it sometimes happens that the supposed mole parent itself grows and forms the protoperithecia which are then fertilized by the putative female. This can obscure the tests for female fertility which might otherwise be relatively clear. One way to get around this problem is by using female sterile strains as the fertilizing parent in a cross. However, a few female sterile strains have been tested here for use as male parents in test crosses. All were found to give lower levels of fertilization than wild type. The per mutants described here show no lowered level of ability to function as the mole parent in a cross. If the per parent functions as the female this can be immediately seen because the perithecia are yellow instead of block. Thus the genetic identity of the mole and female parents can be determined by inspection.

Many female sterile strains can be helped through a cross as the female parent if they are put into a heterokaryon with a female fertile strain. This heterokaryon is then used as the female parent. A number of investigators have used this method of crossing female strains. per female fertile/per+ female sterile heterokaryons offer on additional convenience if female sexing is desired. The ascii in which the per nuclei of interest are participating can be recognized because they segregate 8:0 instead of the 4:4 spore color pattern of per. By coupling appropriate biochemical markers to the per nucleus and/or by using the per as the male parent in the cross also, random spores from the desired mating can be isolated from the same cross.

per might also be convenient for studies of interallelic recombination. Apparent interallelic recombination has been observed in crosses of various per alleles. The block ascosporangia provide a very easy means of detecting recombination which could be used with random spores or with intact ascii. The inability of per spores to withstand heat shock would serve as an additional means of recombinant selection.

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Chalmers, J. H. Toxicity of antibiotics and other drugs to Neurospora.

In a search for agents suitable for isolating cytoplasmically-inherited drug-resistant mutants, a number of antibiotics, antibacterials, respiratory inhibitors and compounds known to induce the “petite” phenotype in yeast were examined. Toxicity tests were performed in 2 ml of Vogel’s Medium N in 4-inch stationary test tubes. Sucrose at 2% (w/v) was used as a fermentable carbon source; sodium acetate at 40 millimolar or glycerol at 2% was employed as a non-fermentable substrate. The pH of the drug-containing media was either pH 5, 6.5 (low pH) or pH 7.5-8.5 (high pH), and the growth rate of 74A was found to be reasonably good over this entire range. Germinating conidio of five-day-old cultures of 74A were added to a final concentration of approximately 10^6 conidia/ml, and the cultures were incubated at 34° for 5 days. The concentrations of the drugs given in Table 1 [following page] are either those which permitted no visible growth in 5 days, or the highest concentrations which, for technical reasons, were tested.

In general, aminoglycoside antibiotics are ineffective, except for kasugamycin and poromomycin. Macrolides are toxic only at the higher pH and show little discrimination between fermentable and non-fermentable carbon sources. In fact, a good portion of the differences seen between the two carbon sources and the two pH’s is probably due to the differences in growth rate observed under these conditions in the absence of any drugs.

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The symbols and abbreviations used in the table on the following page are given below:

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Molar 
No measurement 
Amount of drug/ml of medium 
Growth still observed at this conc 
Approximate limit of growth 
Solubility limit of drug 
Drug is unstable 
Microgram