A Study of the Abnormal-1 and the Poky Strains of *Neurospora crassa* for Complementation

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ABSTRACT

An investigation to determine if 2 slow-growing cytoplasmic, respiratory deficient mutants, poky and abnormal-1, inositol, would show complementation when mitochondria carrying these mutant traits were brought together in the same cytoplasmon. To do this, it was first necessary to cross poky to an auxotroph, p-aminobenzoic acid, to obtain the double mutant, poky, p-aminobenzoic acid. Poky, p-aminobenzoic and abnormal-1, inositol were then placed on a minimal medium on which neither would grow alone, but growth would only occur as the result of the fusion of hyphae. This would determine whether a heteroplasm could be formed and whether complementation could occur at the cytoplasmic level. A series of controls were established to show if any increase in growth rate was due to heterosis between the nuclear components.

Measurement of the growth rate of the heterokaryotic homoplasmon clearly showed no increase in the growth rate, and indicated that complementation did not occur between the poky and abnormal-1 mitochondria.

INTRODUCTION

Bertrand and Pittenger (1972a) advanced a scheme for the classification of all the known extranuclear cytochrome deficient mutants in *Neurospora crassa*. This classification is partially based on the ability of members of 3 groups in this classification to show complementation only with members of another group in heteroplasmosis. This has been shown to be true in all cases investigated to date, except one (Bertrand and Pittenger 1972b). Two members of the third group, abnormal-1 and abnormal-2, have not been tested. It was the purpose of this paper to determine if there was complementation between abnormal-1 and a member of the first group (poky).

MATERIALS AND METHODS

Various media were used according to the growth requirements and the type of spore production desired. All cultures were maintained in stock on BBL *Neurospora* culture agar except for abnormal-1 which was maintained on plates of BBL potato dextrose agar. Difco *Neurospora* minimal medium with 2 percent BBL granulated agar was used for measuring growth rates and forcing heterokaryons, except where otherwise indicated. Difco Cornmeal agar (B 386) was the medium for all crosses.

The standard St. Lawrence strains of *N. crassa* 74 *A* and 77 *a*, were obtained from Dr. K. E. Papa of the University of Georgia. An auxotroph for inositol with mating type *A* was acquired from Colorado State University. The Fungal Genetics Stock Center supplied the following strains: p-aminobenzoic acid (pab-1) No. 1633 *A* and *a*, inositol (inos) No. 37401 *a*, poky (mi-1)mi-1-1.8 *a*, and abnormal-1 inositol (abn-1, inos) No. 37401 *a*. Strains, poky aminobenzoic and poky inositol, could not be obtained from any source and were produced by crossing (Mitchell et al., 1953). A poky (pab-1) mutant with type *a* mating type and a poky (inos) mutant with mating type *a* were then isolated.

The cytoplasmic genetic determinants are given in brackets, e.g., [poky], with the nuclear genotype given in parentheses, e.g., (pab-1) and therefore (pab-) [poky] would mean the strain is a nuclear mutant for aminobenzoic acid and also has the cytoplasmic poky growth trait.

A series of controls would be necessary (Pittenger 1956, Bertrand and Pittenger

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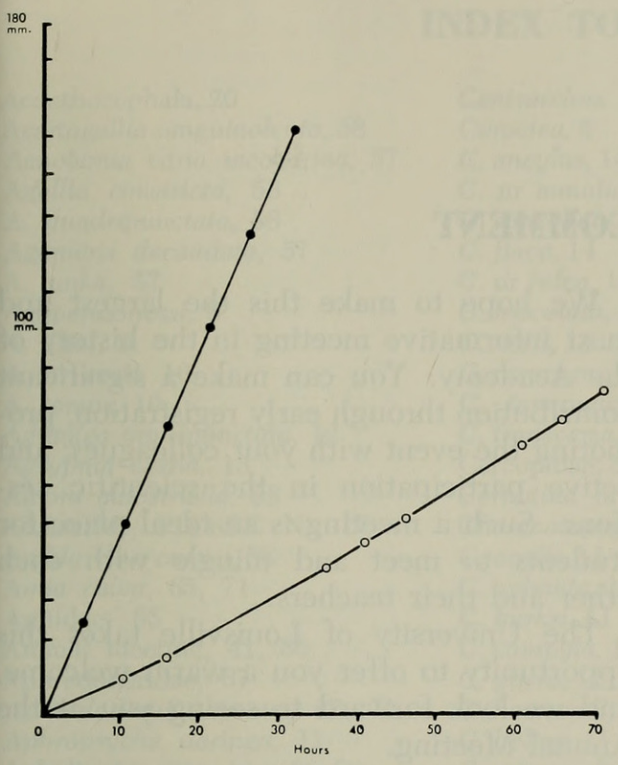

FIG. 1. The growth rates of 77 *a* (●) and (inos) [abn-1] *a* + (pab-1) [poky] *a* (○) on *Neurospora* minimal medium at 35 C.

1972b) if complementation was observed to prove it was due solely to cytoplasmic interaction.

The linear growth rate for this study was determined by growth tubes modelled after the ones used by Ryan et al. (1943) with slight modifications.

In measuring growth rates, the growth tubes were inoculated with conidia and (or) hyphae of the desired strain or strains of *N. crassa*. The growth tubes were allowed to incubate at 35 C for 24 hours and then the mycelial frontier was marked on the glass tube with a felt pen. The position of the advancing mycelial frontier was marked at regular time periods afterward, and the growth rate was measured in millimeters per hour. The normal growth rate of *N. crassa* at 35 C was determined by measuring the growth rate of 77 *a*.

RESULTS AND DISCUSSION

The growth rate for 77 *a* (Fig. 1) was 4.99 mm per hour on minimal medium. The growth rate of the heterokaryotic heteroplasmon (pab-1) [poky] *a* + (inos) [abn-1] *a* (Fig. 1) averaged only 1.44 mm per hour. This indicates that complementation occurred on the nuclear level since neither of these auxotrophs would grow on minimal medium alone, but the slow growth rate means that complementation has not occurred on the cytoplasmic level.

These results indicate that complementation between poky and abnormal-1 does not occur with the method applied in this study. This indicates that each one of these mutations does not represent a part of different mitochondrial cistrons.

While poky has shown complementation with other cytoplasmic mutants, abnormal-1 has not. The apparent failure of it to show complementation with poky in this study indicates the need to know if it will show complementation with any of the other cytoplasmic mutants.

LITERATURE CITED

BERTRAND, H., AND T. H. PITTENGER. 1972a. Isolation and classification of extranuclear mutants of *Neurospora crassa*. Genetics 71: 521–533.

————, AND ————. 1972b. Complementation among cytoplasmic mutants of *Neurospora crassa*. Molec. Gen. Genet. 117:82–90.

MITCHELL, M. B., H. K. MITCHELL, AND A. TISSIERES. 1953. Mendelian and non-Mendelian factors affecting the cytochrome system in *Neurospora crassa*. Proc. Natl. Acad. Sci. USA 39:606–613.

PITTENGER, T. H. 1956. Synergism of two cytoplasmically inherited mutants in *Neurospora crassa*. Proc. Natl. Acad. Sci. USA 42: 747–752.

RYAN, F. J., G. W. BEADLE, AND E. L. TATUM. 1943. The tube method of measuring the growth rate of *Neurospora*. Amer. J. Bot. 30: 784–799.