Fungal Genetics Reports

Volume 7 Article 9

Induction of microconidiation and macroconidiation on the same Neurospora stock

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Abstract
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Special strains. Microconidia have some distinct disadvantages when used in mutation experiments. These include the uncertainty of predicting the size of viable populations or the inconvenience of testing microconidial cultures. In general macroconidia do not suffer from these limitations. We have used two stocks which could be induced to produce either microconidia or macroconidia at will by varying the environment in which they were grown and which have been convenient to use in mutation experiments. They both were derived from a microconidiate pe; col-1 culture obtained originally from Stanford.

One, pe, su-pe; col-1; acon-t (called cms 22), was picked up in a culture of the pe;col-1 stock (Grigg 1958 J. gen. Microbiol. 19:15; 1960 J. gen. Microbiol. 22: 662 and 667) It is colonial in growth habit but macroconidiate at 25°C. cms 22 is aconidiate at 35°C and if macroconidiation is prevented by keeping the freshly inoculated cultures at this temperature for 2 days or longer or by submerging the culture for a similar period at 25°C it produces microconidia almost exclusively (99%) when moved to an environment where conidiation is again permitted. Microconidiation was best on Westergaard and Mitchell's (W and M) crossing medium at pH 5.6 supplemented with casein hydrolysate (1g./l.). As an example of the yield of conidia to be expected, in one trial the following yields of microconidia were obtained, 7 ml slope cultures being used throughout: W and M minimal, $5.1 \pm 1.0 \times 10^7$/tube; casein hydrolysate supplemented medium, $3.0 \pm 0.5 \times 10^8$/tube. The yield on Fries medium or on W and M medium at pH 6.5 was rather less. On rich complete media, e.g., Horowitz's conidiating medium, the stock was macroconidiate at all temperatures.

The other micro/macroconidial stock which we have used is cm 9, obtained as a segregant from a cross of cms 22 with a sfo strain. It had the genotype pe, su-pe$^+$; col-1; acon-t. cm 9 was normally microconidiate at 25°C and macroconidiate at 35°C. It could be induced to produce microconidia at 35°C, however, by keeping cultures at 25°C for 3 days and then transferring them to 35°C. Microconidia appeared about 3 days later. The yield of conidia in this strain was about the same as in cms 22. Usually, viability of the microconidia in both stocks was 10%. The macroconidia produced by both cm 9 and cms 22 cultures were slightly smaller, more uniform in size and had slightly fewer nuclei than wild type strains. In these character-

istics they closely resembled clumpy (pe; col-I$^+$) macroconidia.

Inducing microconidiation in a normal macroconidial strain. Microconidiation of stocks which carry none of the mutant genes necessary to cause microconidia production, such as pe; fl or pe; col-I, can be achieved by synthesizing forced heterocaryons with cms 22, making the nuclear ratio between 20 and 40 to 1 in fav of cms 22. As an example we constructed a heterokaryon between cms 22 and a macroconidial stock which carried the markers al-2 and hist-3. The heterokaryon, which had a nuclear ratio cms 22: hist-3, al-2 of 30:1 produced microconidia copiously over the non-colonial culture on conidiating medium. It may be noted that here we have two strains each of which are always macroconidiate on this medium but form a heterokaryon which is microconidiate.

This sort of technique for inducing microconidiation should be applicable to other pe$^+$ strains.

Problems of using cms 22. At least 4 genes are involved in controlling its conidial character. Mutation of any one leads to loss of ability to produce either or both macroconidia and microconidia, col-I being particularly prone to mutate to a col-I$^+$ state (Barratt and Garnjobst 1949 Genetics 34: 351). As a consequence the culture must be continually reisolated. As well, its fertility has always been poor and inclined to be irregular. - - - C.S.I.R.O., Division of Animal Genetics, Sydney, N.S.W., Australia.