Wickramaratne, M. R. T. A method for selecting linked markers in Sordaria brevicollis.

In conversion studies "sing spore colour mutants it is useful to have linked morphological or biochsmicol markers flanking the mutant under study. A modification of the method devised by Loin, Mitchell and Houlahan (1948 Genetics 34: 435) for Neurospora was used to obtain such markers linked to grey-5 (g-5) which is on linkage group N iin Sordaria brevicollis.

Wild-type strains Rwt1a and Rwt8A and mutant strains B7A and B7a (B7 is an allele of the grey-5 gene) were cultured on crossing medium described by Fields and Olive (1967 Genetics 57: 483). After four days growth, microconidia from each wild-type strain were suspended in sterile distilled water and exposed to 4 minutes UV irradiation. These treated suspensions were poured onto the protoperithecial cultures of B7 strains of opposite mating-type, left for about 5 to IO minutes and poured off. The crosses were incubated at 25°C in the dark and matured in one week.

Ascospores were collected by placing petri dish lids containing special germination medium (sucrose 30 g, glucose 209, sorbose 2 g, sodium acetate 1 g, Difco Bacto agar 40 g, water up to 1 litre) over the open crossing plater, in the light. The lids were replaced with new ones after about 5 to 30 minutes to avoid overcrowding the octads of discharged spores on the agar surface. These lids were incubated overnight at 25° C.

The lids containing germinating spores were scanned under the dissecting microscope. Certain groups of octads hod four spores germinating normally with the other four growing more slowly: these slow-growing spores were possible mutants induced by uv. A search was made for octads where the putative mutants were all or mainly black spores. These spores were isolated and tested on crossing medium and on minimal medium (glucose 20g, Difco Bacto agar 15g, Westergaard salt solution 100 ml, water "p to1 litre). Cultures which grew on crossing medium but not on minimal medium were tested for nutritional requirements by inoculating them on minimal agar supplemented with various combinations of amino acids, vitamins, purines and pyrimidines (Holliday 1956 Nature 178: 987). Those which grew on minimal medium were discorded if growth were normal but subcultured if growth were colonial, morphological or slow. All mutant strains were backcrossed to wild-type to check stability of the mutants and re-isolated mutant strains were added to the mutant collection. These were then crossed by B7 to test for linkage.

Results are summarized in Table 1. Out of a total of 16 mutants obtained, 9 were linked to g-5; this is a yield of over 50%. Other methods were found to be less efficient. For example, uv irradiation of mutant spores (g-5) either killed the spores or left them unaffected. The filtration enrichment method (after Fries method of Ophiortoma; 1947 Nature 159: 199) was unsuccessful due to the poor germination of ascospores in minimal medium and the clogging of the pores of the filter by growing hyphae. It may be possible to "se the special germination medium used here, which greatly improver percentage germination of ascospores, in liquid form; this was not attempted as this medium was discovered only after filtration enrichment was abandoned as being unsatisfactory.

Table 1. Selection of morphological and biochsmicol markers in Sordaria brevicollir.

| cross* | plate designation | number of spores picked[Δ] | number of cultures obtained | number of morphological mutants | biochemical mutants: number | biochemical mutants: requirement | number linked to g-5 |
|---|---|---|---|---|---|---|---|
| B7A x Rwt1a | D | 28 | 10 | 3 | 4 | methionine, pyridoxine, tryptophan, unknown | 4 |
| | B | 20 | 6 | 3 | 1 | methionine | 2 |
| | A | 7 | 4 | 1 | 0 | | 1 |
| B7a x Rwt8A | C | 3 | 2 | 1 | 1 | riboflavin | 2 |
| | E | 5 | 3 | 2 | 0 | - | 0 |
| | F | 1 | 1 | 0 | 0 | - | 0 |
| | Total | 64 | 26 | 10 | 6 | | 9 |

'Crosser "ring Rwt1a were more fertile, possibly because this strain produced more microconidia than Rwt8A.

*Each perithecium usually sheds spores into a restricted region. The number of spores picked indicates a single spore from each such region.

The method described here was also carried out using wild-type strains only. This yielded some mutants, both morphological and nutritional, but the number linked to g-5 were, of course, proportionally less: of nine morphological mutants two were linked to g-5, one very loosely, and of four nutritional mutants (requiring pantothenic acid, pyridoxine, valine and with on unknown requirement) one was linked to g-5. This is a little over the expected one in seven, considering that Sordaria brevicollis has seven linkage groups. From crosses of B7 to wild-type where there woo no conscious selection, two morphological mutants unlinked to g-5 and one nutritional (requiring on unknown amino acid) linked to g-5 were obtained. Using a lower dosage (60 seconds uv irradiation) ond other wild-type strains, one morphological and one nutritional (pyrimidine-requiring) mutant were obtained.

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