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Abstract
Heterothallic species of Neurospora are distinguishable by restriction analysis of their nuclear rDNA sequences.

This research note is available in Fungal Genetics Reports: https://newprairiepress.org/fgr/vol30/iss1/1
Heterothallic species of Neurospora are distinguishable by restriction analysis of their nuclear r-DNA sequences. Several recent reports have shown that restriction analysis of mitochondrial and chloroplast DNA's have been used successfully to distinguish species differences for phylogenetic studies in plants and animals. We have tested this approach of restriction analysis of r-DNAs to distinguish nuclear rDNA's of three different reference strains of heterothallic species of the genus Neurospora: N. crassa 74A (FGSC #987), N. intermedia PA420 (FGSC #2316), and N. sitophila I0B (FGSC #580). Two approaches were adopted: (1) Nuclear DNA's of these three Neurospora species were treated with various restriction enzymes. Against the streaks of nuclear DNAs on the 0.7% agarose gels background bands were visible. These background bands are visible because rDNA sequences of Neurospora species exist in multiple copies within the nuclear DNA's (Chathopadhyay, Kohne and Dutta, 1972 Proc. Natl. Acad. Sci. USA, 69: 3256). (2) The second approach was comparison of autoradiographs of hybrid molecules of Southern blot transfers of restricted nuclear DNAs and 32P-labelled nick translated rDNA's (referred to as rDNA probe) isolated from N. crassa slime mutant (FGSC #1118), rDNA cloned into pBR322 (the plasmid pMF2, obtained from R.L. Mettenberg, University of Wisconsin, Madison). This 10.4kb rRNA probe contains 4.4 kb of E. coli plasmid pBR322 DNA and all of the 17S and 25S rRNA coding sequences, and the internal spacer region which includes the 5.8S rRNA coding sequences. but lacks the 2.6 kb size external spacer region of N. crassa.

Nuclear DNA's from mycelial of all three Neurospora species were isolated as described by Hautala et al. (1977 J. Bact., 130: 706). Restriction enzymes used were EcoRI, BamHI, HindIII, BglII, Smal, and PstI. All of these restriction enzymes were obtained from the Bethesda Research Laboratory (BRL), Gaithersburg, MD. The reaction conditions and buffers for each restriction enzyme were as described by BRL. The procedures for DNA gel electrophoresis, and autoradiographs of Southern blot transfers were standard (see, for example, the book entitled 'Molecular Cloning' by Maniatis et al., 1982, Cold Spring Harbor Laboratory, N.Y.)

Results obtained so far are briefly summarized in Table I. The restriction enzyme EcoRI generated three bands: 3.1 and 2.1 kb fragments (which were present in all three species) and a 3.4 kb fragment in N crassa only. This 3.4 kb fragment contained mostly external spacer regions. HindIII generated more than one band in all three species; for example, two bands in N. crassa a doublet of 4.0 kb size in N. intermedia, and two bands in N. sitophila. PstI generated one common fragment of approx. 6 kb size in all three species containing rRNA coding regions. In addition, another fragment of approx. 2.3 kb to 2.6 kb size was present in all three species. This indicated that coding regions of rDNAs in all three species were similar, if not identical. When the other three enzymes BamHI, BglII and Smal were used only one band of the nuclear DNAs of each species could be seen in our gel preparations. It is know (Free et al. J. Bact. 137: 1219-1226, 1979) that the enzyme Smal generates 5 fragments (one large and four small) and the enzyme BamHI generates 2 fragments (one large and one small). Apparently, the smaller fragments had run off our 0.7% agarose gels preparations and thus could not be seen.

This investigation was intended to study whether restriction analysis of rDNAs could pinpoint differences among reference wild-type Neurospora species. As a part of our future plans we plan to compare restriction analysis of different isolates within the same species. Extensive studies on DNA homologies (Dutta, 1976...)

### Table I

Summary of restricted fragment sizes as seen in the gels and in autoradiographs of Southern blots of the respective gels

| Neurospora Species | Restriction enzymes: Fragment sizes in kb (1 kb = 1000 bases) |
|--------------------|-------------------------------------------------------------|
|                    | EcoRI | HindIII | PstI | BamHI | BglII | Smal |
| N. crassa          | 3.4, 3.1, 2.1 | 4.9, 3.7 | 6, 2.6 | 8.3 | 8.6 | 5.9 |
| N. intermedia      | 3.1, 2.1 | 4.0 (doublet) | 6, 2.3 | 8.3 | 8.3 | Over 6 |
| N. sitophila       | 3.1, 2.1 | 4.5, 4.0 | 6, 2.5 | 8.3 | 8.5 | 5.9 |

High molecular weight nuclear DNAs were isolated from mycelial cells of all species. In general 1 µg of nuclear DNA was restricted with 1-2 units of the respective enzymes. Each of the nuclear DNAs was restricted at least three times along with the (lambda) and pMF2 DNAs as controls for estimates of fragment sizes in kb (1 kb = 1000 bases). Lambda DNA was restricted with HindIII because it generates 23 kb to 100 bp size fragments for each experiment and pMF2 DNA was restricted with the same enzymes as the nuclear DNAs of each Neurospora species.
During the last several years we have isolated and worked with a number of siderophore transport mutants (sit) in Neurospora crassa. The work is unfinished and nothing has been published about these mutants, except for their listing in the "Compendium" (Perkins, et al., 1982 Microbiol. Rev. 46: 426). Since one of us (GWC) is no longer working in this field, we have decided to make the mutants available to anyone interested. This note introduces the mutants, their background, isolation and general characteristics. They have been deposited in the Stock Center (FGSC4211 through 4231) in the current stock list.

**Background:** The parent strain is the triple mutant, arg-5, ota, aga (FGSC 2744), which was obtained from R.H. Davis. This mutant is blocked in all known pathways of ornithine synthesis and produces so little ornithine (Charlang and Ng, 1982 Neurospora News 29: 15) that it becomes siderophore-dependent when grown in medium with glycerol as carbon source. This observation formed the basis for use in "the hunt for" siderophore transport mutants.

**Isolation:** To reduce siderophores pools in conidia, the triple mutant was grown without ornithine prior to the "hunt." UV-treatment of conidia was followed by filtration enrichment in glycerol medium supplemented with ferricrocin (FC) (Vogel's N-free salts; glycerol and asparagine). All media contained putrescine and arginine (recrystallized) which were filter-sterilized and added to the autoclaved medium. All liquid media also contained Tween 80 [four drops (56 mg per 100 ml)]. FC was added to the autoclaved medium at 10 to 20 µg per 100 ml. Ungerninated conidia were plated on sorbos medium supplemented with ornithine. One thousand isolates from these plates were tested for growth in liquid glycerol medium (same medium as above, except Winkelmann's salts (Winkelman and Zahn 1973 Arch. Microbiol. 88: 49) instead of Vogel's, with trace elements added (but not Fe or citrate) supplemented with either ornithine or FC. Those 120 isolates that grew on ornithine but not on FC were tested for 3HFC uptake. The medium used in the uptake was the liquid glycerol medium (Winkelman's salts) used in the growth tests of the original isolates. Conidia were germinated for 3 hours at 30°C before measuring uptake.

Eleven isolates were found to be uptake deficient. To check specificity of the uptake defect they were tested for 13C-phenylalanine transport. Six of the 11 isolates appeared to have a general transport defect as they were also deficient in phenylalanine uptake. They have been set aside and not analyzed any further. (They are not in the Stock Center but GWC still has silica gels, if anyone would like to have them.) The remaining five isolates were normal as far as phenylalanine transport is concerned, and they were designated as sit mutants.

**Crosses:** The five sit mutants were crossed to 74a in order to remove the parental marker genes. Progeny were first analyzed in low water activity (aw) medium with or without ferricrocin. This test was based on our earlier finding that, in low aw media (aw < 0.93), conidia lose that part of their ferricrocin pool which is required for germination (Horowitz et al., 1976). Bacteriol. 127: 135). Germination will occur, however, if the medium is supplemented with the siderophore. Uptake mutants should show little or no response to added ferricrocin. Before these crosses were completed others were set up between sit mutants to test for allelism. The five mutations appear to be nonallelic. (Some of the double mutants obtained at that time have been deposited in the Stock Center. Many still carry the arg-5 genotype and may be of limited usefulness in siderophore transport studies.)

The mutations have not been mapped.

**General Description:** The sit mutants grow well on minimal medium. sit-1 germinates slowly. sit-2 has a deep orange color, and its conidia tend to remain connected in twos or threes. sit and sit-4 are very poor protoperithecial parents.

None of the mutants is totally unable to transport FC or coprogen. There is some passive FC and coprogen uptake in Neurospora, and this may be what we are observing in these mutants. sit-1 and sit appear to be binding mutants. Their ability to bind FC to the cell surface (in presence of NaN₃ which inhibits active transport)