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Heterothallic species of Neurospora are distinguishable by restriction analysis of their nuclear r-DNA sequences.

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Abstract

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RESEARCH NOTES

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Several recent reports have shown that restriction analysis of mitochondrial and chloroplast DNA's have been used successfully to distinguish species differences for phyloqenetic studies in plants and animals. We have tested this approach of restriction analysis of rDNAs 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 Neursospora 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 auto-radiographs of hybrid molecules of Southern blot transfers of restricted nuclear DNAs and ^{32}P-1abelled 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. Metrenberg, University of Wisconsin, Madison). This 10.4kb rDNA probe contains 4.4 kb of E. coli plasmid pBR322 DNA and all of the 17S and 26S rRNA coding sequences, and the in the internal spacer region which includes the 5.8S rRNA coding seqences. 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, SmaI, 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 SmaI 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 SmaI 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	SmaI
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 Hind111 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.

Mycologia LXVIII: 388-401) of numerous isolates of the species N. crassa did not show any significant variability in non-repeated DNA sequences. Recent studies on restriction analysis of rDNAs of different cell types of N. crassa (Chambers and Dutta, unpublished) have also clearly shown no variability within the rDNAs of the same species. Hence, it is obvious that restriction analysis of rDNAs is a reliable means to study genetic characteristics, and should prove to be a very useful tool for identification of species of eukaryotic organisms. (Supported in part by a Department of Energy Grant.) - - - Department of Genetics and Botany, and Cancer Research Center, Howard University, Washington, D.C. 20059.