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Characterization and measurements of nucleotide sequence similarities of DNA's from several *Neurospora* species.

Highly purified DNA in large quantities can be isolated easily from *Neurospora* mycelia using hydroxyapatite chromatography, as described by Chattopadhyay and Dutta (1969 *Neurospora News* 15: 11). We have prepared mycelial DNA from wild type strains of four species of *Neurospora* (*N. crassa*

74A, *N. intermedia* 10B A, *N. sitophila* 56. 10 and *N. tetrasperma* 85A, all obtained from the Fungal Genetics Stock Center), one mutant strain of *N. crassa* (slime, sl, obtained from V. W. Woodward), and some natural isolates of undetermined species (Gianfor 1A/a, Kuala Lumpur 1e a, Mysore 1e a, Obama 1b o, and Lahore 1A) all obtained from D. D. Perkins.

All of these DNA preparations show two components. The major component, which has a high G + C moles percent (51 to 54 %), accounts for approximately 70% of the total DNA isolated. The minor component has a G + C moles percent of 32 to 37%. These moles percent G + C values were calculated on the basis of Tm values obtained from thermal profile curves, using a Gilford recording spectrophotometer. A comparison of the profiles obtained, by plotting percents of total hyperchromicity against temperature, of DNA's from different *Neurospora* species revealed some differences. Such differences are not detectable when mycelial and conidial DNA's isolated from the same species (*N. crassa* 74A) are compared.

DNA:DNA hybridizations were performed, using the method of Britten and Kohne (1968 *Science* 161:529), between ³²P labeled DNA of *N. crassa* (192,000 cpm/μg DNA) and unlabeled DNA from other *Neurospora* species. These preparations, giving a minimal total Cot (OD per ml/2 x hrs. of incubation) of 1500 in 0.14 M phosphate buffer at pH 6.8, showed a percent homology that varied from 75 to 85 %. The *N. crassa* ³²P alone that was used in these reactions was given a negligible Cot to minimize self-reaction. The table below summarizes some of these hybridization data.

Table 1. Summary of homologous and Heterologous DNA:DNA reactions among *Neurospora* species.

Labeled DNA fragments	Unlabeled DNA fragments	Percent Homology* Measured (average)	Normalized	Tm of labeled material	Tm**
<i>N. crassa</i>	<i>N. crassa</i>	94.6	100	90.5 °C	0.3 °C
"	<i>N. intermedia</i>	79.8	84.4	85.5	5.1
"	<i>N. sitophila</i>	83.7	80.5	85.2	4.3
"	<i>N. tetrasperma</i>	80.5	85.1	86.0	5.5
"	<i>Coprinus lagopus</i>	18.6	19.7		
"	<i>E. coli</i>	0.4	0.42		

• Reactions of homologous DNA's of *N. crassa*, *N. intermedia*, *N. sitophila* and *N. tetrasperma* were 94.3, 96.3, 97.9 and 90 %, respectively. The average value of 94.6 = 100% binding was used for normalization of the heterologous reaction data above.

• Tm was determined by comparing the Tm of the radioactive elution profile with its corresponding optical density data.

All of these *Neurospora* DNA preparations were hybridized with ^{32}P labeled DNA (4800 cpm/ μg DNA) from a distantly related fungus, *Coprinus lagopus* H₂. The results obtained showed a range of 12 to 25 % hybridization at a total Cot of 1224. Self-hybridization of ^{32}P *C. lagopus* DNA at a Cot of 0.45 to 0.56, used in these reactions, was 2.7 to 3.5 % for which the necessary corrections were made in the calculations. Using labeled *N. crassa* DNA, a net hybridization of 12.5% was obtained, at a total Cot of 261.1. When labeled *C. lagopus* DNA was hybridized with unlabeled *C. lagopus* DNA, using a Cot of ca. 500 in both cases, more than 95% hybridization was obtained. Similar hybridization between ^{32}P labeled *C. lagopus* DNA (giving a very low Cot of about 0.3) and unlabeled DNA from the procaryote *E. coli* (giving a very high Cot of more than 500) gave only 0.75% hybridization.

The different values obtained for percentage of homologous sequence do not permit us to establish precise genetic inter-relationships among *Neurospora* species. The T_m 's which we have obtained with the heterologous DNA:DNA interactions (unpublished results) indicate, however, that the species *N. intermedio*, *N. sitophila* and *N. tetrasperma* are apparently more or less equally distantly related to *N. crassa*, differing by 3 to 7% nucleotide sequences (1°C T_m difference = $\pm 1\%$ DNA sequence difference). Possibly all of these four *Neurospora* species diverged from a common ancestor. Similar studies are underway, using labeled DNA of *N. intermedio*, to confirm these conclusions.

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