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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*l. 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 (*Gianjor* 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 sequencer 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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