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Abstract
Characterization of DNA's from several Neurospora species

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

Highly purified DNA in large quantities con be isolated easily from Neurorporo mycelia using hydroxyopotite chromatography, as described by Chattopadhyay and Dutta (1969 Neurospora Newsl. 15: 11). We hove prepared myceliol DNA from wild type strains of four species of Neurorporo (N. crassa 74A. N. intermedio 10B A, N. sitaphilo 56. lo and N. tetrasperma 85A, all obtained from the Fungal Genetics Stock Center), one mutant strain of N. crassa (slime, sl, obtained from V. W. Woodword), and some natural isolates of undetermined species (Gianjor 1A/a, Kuala Lumpur 1e a, Mysore le a, Obama lb o, and Lahore lA) all obtained from D. D. Perkins.

All of there DNA preparations show two components. The major component, which hor 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 spectraphotometer. 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 ore not detectable when myceliol and conidiol DNA's isolated from the same species (N. crassa 74A) ore compared.

DNA:DNA hybridizations were performed, using the method of Britten and Kohne (1968 Science 161:529), between ^{32}P labeled DNA of N. crassa (192,000 cpm/µg DNA) ond 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 phorphote buffer at pH 6.8. showed a percent homology that varied from 75 to 85 %. The N. crassa ^{32}P 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**
N. crassa	N. crassa	94.6	100	90.5 °C	0.3 °C
" "	s intermedia	79.8	e4.4	85.5	5.1
" "	N. sitophila	83.7	80.5	85.2	4.3
" "	N. tetrasperma	80.5	85.1	86.0	5.5
" "	Coprinus lagopus	18.6	19.7		
" "	E. coli	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 comporing 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 H_2. 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 there 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 1. When labeled C. lagopus DNA was hybridized with unlabeled C. lagopus DNA, using a using a Cot of co. 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 interrelationships among Neurospora species. The Tm's which we hove obtained with the heterologous DNA:DNA interactions (unpublished results) indicate, however, that the species N. intermedio, N. sitophila and N. tetrasperma ore apparently more or less equally distantly related to N. crassa, differing by 3 to 7% nucleotide sequences (1°C Tm difference = ± 1 % DNA sequence difference). Possibly all of these four Neurospora species diverged from a common ancestor. Similar studier are underway, using labeled DNA of N. intermedio, to confirm these conclusions.

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