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Factors affecting DNA uptake by Neurospora crassa

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Abstract

Factors affecting DNA uptake

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Factors affecting DNA uptake by Neurospora crassa.

It was described earlier (Szabo and Schablik 1973 Neurospora Newsl. 20:27) that N. crassa cells are capable of taking up exogenous DNA. We have recently isolated ^{3}H-labelled DNA of an average molecular weight of 30×10^6 from N. crassa. The effect of various experimental conditions on DNA uptake, i.e. Ca^{2+} concentration, pH, and denaturation, is described here.

N. crassa RL-3-8 (inl', rg', A) was grown on Vogel's minimal medium supplemented with ^{3}H-adenine (5.5 µCi per ml) and the labelled DNA was isolated. From a 2.25 liter culture, 20-24 g wet mycelia were obtained and washed with 0.01 M EDTA in 0.08 M phosphate buffer, pH 8. Then the mycelia were homogenized at -30° C and the DNA was extracted by the modified Marmur's method (Marmur 1961 J. Mol. Biol. 3:208; Hansen 1974 Preparative Biochemistry 4:473). The crude DNA was passed through a Sepharose 2B column in 15 mM NaCl, 0.15 mM citrate pH 7.0. High molecular weight DNA fractions were collected and concentrated by vacuum dialysis. The average molecular weight of DNA determined by sucrose-gradient centrifugation was 30×10^6.

The recipient N. crassa R2506-5-101 (inl, rg, a) strain was grown on Vogel's minimal medium containing 100 µg/ml inositol. After 20 hours incubation in a rotatory shaker the mycelia were harvested and washed to remove extracellular nucleases.

The pH of 3 ml samples of mycelia in fresh medium was adjusted by the addition of 0.5 ml buffered solutions. To each, DNA was added to a final concentration of 5 µg per ml. Cultures were shaken for 30 min at 28° C and then were chilled to 0° C and centrifuged. The mycelia were resuspended in 1 ml 50 mM acetate and 5 mM $MgCl_2$, which contained 1 mg DNase (DNase I, Sigma, 1115 Kunitz units per mg) and was incubated for 5 min at 20° C. Then the mycelia were washed with 2 ml of buffer A (0.4 M NaCl, 0.1 M Na-phosphate, pH 6.0). The DNA content of the mycelia was extracted with 1 ml 0.5 M $HClO_4$ at 90° C and the radioactivity of the extract was determined by liquid scintillation counting. The pH dependence of DNA uptake is shown in Fig. 1 which reveals an optimum at pH 6.4.

The Ca^{2+} dependence of DNA uptake was studied in a similar way at the usual pH of the culture medium, i.e. at pH 5.8. The uptake of DNA increased with higher Ca^{2+} concentrations until a plateau was reached at about 75 mM (Fig. 2). The effect of denaturation of DNA was also studied by the comparison of the uptake of single and double stranded DNA. DNA was denatured by alkali at pH 13 or by heating the DNA solution to 95° C for 5 min, then chilling immediately to 0° C. Uptake of native and denatured DNA (50 µg/ml) was examined with 75 mM Ca^{++}. The rate of the uptake of denatured DNA was significantly higher than that of the native DNA.

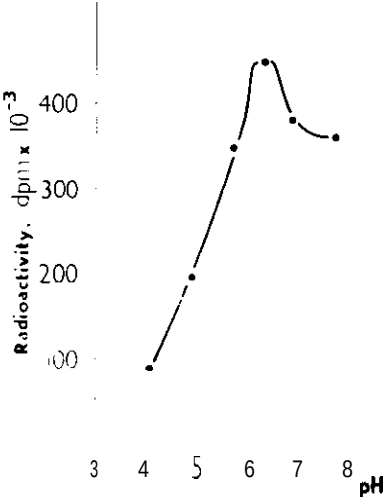

Figure 1. Effect of pH on DNA uptake by Neurospora crassa.

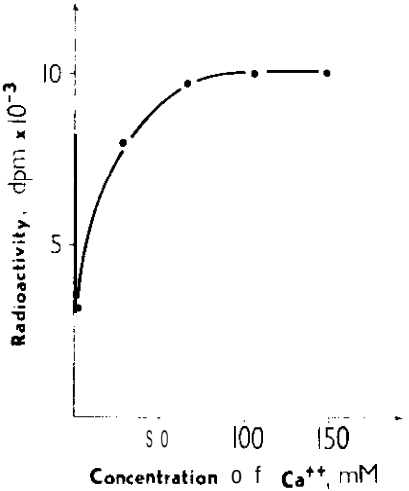

Figure 2. Effect of calcium ion concentration on the DNA uptake.

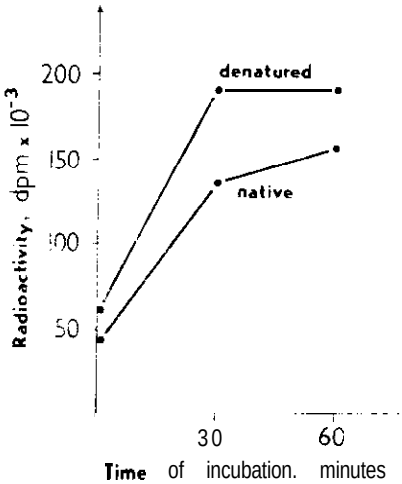

Figure 3

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