Szabó, G. and M. Schablik. DNA uptake by N. crassa.

Genetic changes in N. crosso induced by DNA hove been reported by Szabó, Mishro and Tatum (ot the XVI Ann. Meeting on Microbial Transformation 1972 Estes Pork, Colorado) and by Mishra, Szabó and Tatum (in Niu and Segal, ed 1973 Roles of RNA in development and reproduction, North Holland Pub. Co.

DNA from N. crosso wild type, Rockefeller RL-3-8A, if added to cultures of N. crosso R 506-8-12 inos; rg, increased the number of revertants of the inos character, but only in 37 of the 55 experiments and they appeared in very low frequency compored to transformation in bacteria. It is desirable to find more reproducible conditions of obtaining revertants in higher frequency. The frequency of genetic transformation and the effectivity of DNA uptake shows a parallel course in bacilli (Miller et al. 1972 J. Bocteriol. 1 10:661). Therefore, the investigation of conditions influencing DNA uptake by N. crassa was undertaken. In the following, we report results about the uptake of 32P-labeled DNA from N. crosso wild type by the N. crosso inos strain above. The wild-type strain was cultivated at a phosphate concentration of 5×10^{-4} M in minimal medium of Vogel. DNA was prepared by Marmur's method (1961 J. Mol. Biol. 3: 208). The preparation's activity was 6.4×10^3 cpm/µg DNA. The molecular weight of our DNA preparation was estimated to be about $1.15-1.9 \times 10^6$ daltons, according to determinations of its Rf value in acrylamide gel electrophoresis (Gregson 1972 Anal. Biochem. 197:596). The bulk of the DNA consisted of the aforementioned molecular weight, but there were two minor bonds of higher molecular weights of $1.9-3.1 \times 10^6$ and 3.1-5.3 x lob daltons, respectively.

Mycelial fragments of the recipient strain were prepared with a Waring blendor in 0.03 M phosphate buffer, from colonies grown on minimal medium supplemented with 100 mg/liter myo-inositol. They were then cultivated in the some liquid medium in submerged culture. DNA was added to the mycelium in the fresh incubation mixture (minimal medium + inositol). Samples were token at 0, 15, 30, and 60 minutes of incubation. Samples were treated with deoxyribonuclease (DNase I, Koch-Light 30 µg/ml in 5×10^{-3} M $MgCl_2$ for 2 min at 27°C), then extracted four times with 0.5 N perchloric acid (PCA) at 4°C. This

was followed by two extractions with 0.5 N PCA at 70°C for 20 min. DNA uptake war measured by radioactivity in the hot PCA extracts.

DNA “uptake” at “0” time of incubation depends upon the concentration of DNA in the incubation medium. From a DNA solution of 10 µg/ml, 0.2-0.4 µg DNA/mg dry weight of mycelium is taken up at "0" time. This may increase 2-10 fold during 60 minutes of incubation. There are tenfold differences between the values of the absolute amount of DNA taken up at “0” time, depending upon various factors of cultivation and incubation. Therefore, the difference between radioactivity of samples removed after 0 and 60 minutes’ incubation, respectively, is token as the measure of the continuing rate of transport of DNA into the cell interior. DNA uptake is dependent upon the physiologic.1 state of the culture. Mycelial fragments from 7-day-old agar slants or 72-hour-old submerged cultures take up DNA very poorly or not at all. The best uptake was seen with young (20-hour-old) mycelio: on B-IO fold increase of the “zero” minute value was obtained.

There is no continuing DNA uptake at 4°C; sodium azide (10^{-2} M) also blocks DNA uptake. The uptake of high molecular weight DNA is much better than that of its DNase-digested fragments. There is no continuing uptake if DNA digested with DNase (30 µg/ml in 5 x IO-3 M $MgCl_2$ for 30 min) is present at 27°C for one hour in the incubation mixture instead of native DNA. The uptake of high molecular weight DNA is not inhibited by the addition of IO µg/ι ml non-radioactive digested DNA (30 µg/ml DNase, 30 min at 27°C); 75 µg/ml of this preparation is required to decrease intact DNA uptake by about 50%, whereas cold intact DNA molecules, even at such a low concentration as IO µg/ml, decrease uptake to 50% of the control. Increasing the DNA concentration from 0.02 µg/ml to 1.O µg/ml in the incubation mixture led to proportionately higher values of uptake. The pH of the incubation medium influencer DNA uptake. There is no continuing uptake at pH 7.3 or above; on optimum was found between pH 5.75 and 6.7. EDTA does not influence uptake at 10^{-4} M but inhibits it at 10^{-3} M or 10^{-2} M concentrations. Mg^{++} ond Co* ions have a slight enhancing effect upon DNA uptake.

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