Transformation of Nutritionally Deficient Mutants of *Aspergillus niger*

By KALYANI SEN, P. NANDI AND A. K. MISHRA

Department of Microbiology, Bose Institute, Calcutta 9, India

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SUMMARY

Reversions to wild type occurred when conidia of a number of biochemically deficient mutants of *Aspergillus niger* were treated with DNA from the wild-type strain. No reversions or wild-type colonies were obtained when an equivalent number of conidia from deficient strains were either treated with the same DNA as the recipient, or were plated without any DNA treatment. Increase in the percentage of transformation was observed up to 6 µg./ml. of donor DNA. The transforming activity of the donor DNA was found to be inhibited by the action of u.v. radiation, heat and DNase. The frequency of transformation was low which could be attributed to the method of extraction of DNA which involved crushing of cells in the presence of alumina, possibly breaking the DNA into small fragments thus making it biologically less active.

INTRODUCTION

Very little information is available on transformation in moulds. Shamoian, Canzanelli & Melrose (1961) described the transforming activity of a nucleic acid complex in *Neurospora crassa*. Shockley & Tatum (1962) attempted the reversion of biochemically deficient mutants of *N. crassa* through transformation. The results of these authors were not very conclusive in nature. The present authors have tried to demonstrate the presence of the process of transformation in the mould *Aspergillus niger* and have tried to show that reversions occurring in the nutritionally deficient mutants were due to transformation of the mutants to wild type and that this reversion rate was affected if the donor DNA was treated with u.v., heat or DNase.

METHODS

Six biochemical mutants derived through u.v. radiation (Mishra & Nandi, 1959) of a laboratory-isolated strain of *Aspergillus niger*, designated v35 were used as the recipient strains. The strains were 2 *nic*⁻, 3 *lys*⁻, 4 *leu*⁻, 14 *met*⁻, 21 *cho*⁻ and 45 *arg*⁻ requiring nicotinic acid, lysine, leucine, methionine, choline and arginine respectively. Donor DNA was prepared in the following manner: a number of Roux bottles containing 100 ml. of Czapek Dox liquid medium (Clutterbuck, Lovell & Raistrick, 1932) were inoculated with a heavy conidial suspension of v35 and incubated at 28°. A very thin transparent mycelial layer appeared at the surface of the medium in about 24 to 36 hr. This layer was collected by filtration and ground in a mortar in the presence of alumina (mycelium + alumina; 1 + 4) at 5° for about 1 hr. During grinding 10% Na-deoxycholate (0.5 ml./g. mycelium) and citrate saline buffer (1 ml./g. mycelium) were added.

After thorough grinding the mass was finally extracted with citrate saline buffer, from which the protein and RNA were removed by treatment with chloroform and amyl alcohol mixture and RNase respectively. The final DNA preparation which was obtained by precipitation with alcohol was resuspended in 5 ml. of sterile citrate saline buffer and kept at 5°. Purified DNA sample contained about 0.1 mg of DNA per ml. of the solution. The transforming DNA preparations were sterile and did not give rise to any colonies of the donor type when plated alone on Czapek Dox agar medium.

Transformation reaction was carried out in 25 ml. Erlenmeyer flasks by adding 1 ml. of conidial suspension to 8 ml. of Czapek Dox broth, to which DNA solution to make 25 µg/ml had been added. Two controls were usually set up for each lot of experiments. In one control DNA was omitted and, in the other, DNA isolated from the recipient strain was added. The flasks were incubated for 18 to 20 hr at 28° after which the reaction was terminated by adding 0.004 µg. Mg-activated DNase/ml. The transformation mixture was centrifuged and the conidia washed several times before plating on Czapek Dox agar medium. The plates were incubated at 28° for 4 to 5 days when revertants to wild type were observed to appear. Heat inactivation of DNA was done by suspending a number of tubes containing 3 ml. of DNA samples for 30 min at 50°, 60°, 70°, 80°, 90° and 100° in a water bath and u.v. inactivation of DNA was studied by subjecting 5 ml. of DNA solutions contained in a number of 9 cm. plates to u.v. radiations for 2, 4, 6 and 8 min. from a 4 W. General Electric Germicidal lamp (dose incident 100 ergs/mm.²/sec.). After heating and u.v. irradiation the samples were chilled immediately and used for transformation purpose as usual.

Table 1. *Transformation of nutritional markers in Aspergillus niger*

Donor strain—v35, DNA concentration 25 µg./ml.

Recipient strain*	No. of conidia treated†	No. of transformants to wild type			Transformants (%)		
		A	B	C	A	B	C
2 <i>nic</i> ⁻	1.4 × 10 ⁶	0	0	16	0	0	1.1 × 10 ⁻³
3 <i>lys</i> ⁻	3.5 × 10 ⁶	0	0	42	0	0	1.2 × 10 ⁻³
4 <i>leu</i> ⁻	2.7 × 10 ⁶	0	0	15	0	0	5.5 × 10 ⁻⁴
14 <i>met</i> ⁻	3.8 × 10 ⁶	0	0	40	0	0	1.0 × 10 ⁻³
21 <i>cho</i> ⁻	5.5 × 10 ⁶	0	0	162	0	0	2.9 × 10 ⁻³
45 <i>arg</i> ⁻	3.1 × 10 ⁶	0	0	35	0	0	1.1 × 10 ⁻³

* All recipient strains derived through u.v. treatment of v35, for each strain, figure shows the isolate number and symbols denote the requirement as follows: *nic* = nicotinic acid; *lys* = lysine; *leu* = leucine; *met* = methionine; *cho* = choline; *arg* = arginine.

† A = conidia treated with its own DNA; B = conidia not treated with any DNA; C = conidia treated with donor DNA.

RESULTS

Transformation of nutritional markers

Table 1 gives the result of transformation to wild type in a number of deficient strains. Though the frequency of transformation is poor as compared with other transformable organisms, it is apparent that reversions had occurred in the presence of DNA, since controls did not show any revertants. The recipient strains were very stable, no spontaneous reverse mutations could be detected even after plating 1×10^8 organisms on Czapek Dox agar medium.

Table 2 shows that the frequency of transformation increased with the increase in the time of contact of recipient cells with the donor DNA. Germination of conidia started after 8 hr of incubation and by twentieth hr about 95 % had germinated, with number of nuclei varying from two to six per conidium, the majority having two nuclei. In the DNA-treated samples germination was a little better than those without DNA treatment, perhaps due to some helping factors coming through DNA. Bucknall & Morton (1964), while making an attempt to transform *Penicillium chrysogenum*, observed an increase in spore yields and growth rate induced by DNA preparations; however, they were unable to observe the occurrence of transformation. In their

Table 2. Transformation of 3 *cyt*⁻ to wild type

Donor, v35; recipient, 3 *cyt*⁻ (or cytosineless mutant from v35 derived by u.v. irradiation).

Time given for trans- formation reaction	No. of conidia treated* A, B & C	No. of transformants to wild type			Transformants (%)		
		A	B	C	A	B	C
8	3.1 × 10 ⁶	0	0	0	0	0	0
12		0	0	6	0	0	1.9 × 10 ⁻⁴
16		0	0	48	0	0	1.5 × 10 ⁻³
20		0	0	47	0	0	1.5 × 10 ⁻³

* A = conidia treated with its own DNA; B = conidia not treated with any DNA; C = conidia treated with donor DNA.

opinion this stimulation of growth was possibly due to bridging some of the deficiency which might have been in the purine and pyrimidine metabolism of the recipient strain, by utilizing the purines and pyrimidines from the donor DNA. They also suggested the possibility of DNA preparations carrying some trace metals which stimulated growth. A question arises here that in DNA-treated samples there might be an increased probability of occurrence of spontaneous mutations to the transformant type, since these samples were better nourished, but this was not the case, since samples treated with the same DNA as the recipient did not give any revertants. It is possible that the germination helped the cells to become competent and allowed the integration of wild-type growth characters from the donor DNA molecule. In controls without DNA or with own DNA this did not happen since in the former case the samples lacked the donor DNA and in the latter did not carry any marker other than the recipient which could be identified after integration.

To attribute the transforming activity to the donor DNA a number of experiments, reported below, were done to show that transformation was actually taking place.

Transformation at different concentrations of DNA

Transformation experiments were done using different concentrations of donor DNA from v35 varying from 2 to 10 µg. DNA/ml. of transforming mixture. Figure 1 shows the dosage response data as a function of DNA concentration in transforming an arginineless strain (45 *arg*⁻) to wild type. An increase in the percentage of transformation was observed up to 6 µg. DNA/ml. Concentrations beyond that, apparently had very little or no effect on the increase of transformation frequency. This part of the curve could be referred to as the saturation region.

Effect of DNase on transforming DNA

4.0×10^{-3} μ g. of Mg-activated DNase solution/ml. was added to a number of tubes containing 5 ml. samples with 25 μ g. donor DNA per ml. The tubes were incubated at 37°. After suitable intervals of time (Fig. 2), two tubes were taken and EDTA were added to each to a final concentration of 10^{-3} M. Spectrophotometer readings were taken from one and the other transformation experiments were carried out in the usual way. Transformation activity of the donor DNA was found to diminish with each successive increase of incubation time with DNase. Correspondingly an increase in the optical density of the DNA was observed showing the degradation of the DNA (Fig. 2).

Fig. 1

Fig. 1. Percentage transformants as a function of concentration of transforming DNA. (Donor, v35; recipient, 45 *arg*⁻.)

Fig. 2

Fig. 2. Effect of DNase on transforming DNA. (Donor, v35; recipient, 45 *arg*⁻.)

Effect of heat on transforming DNA

Figure 3 shows the effect of heat on transforming DNA. Transformation activity of DNA was found to diminish with the successive rises in temperature (especially at 80° and above). A corresponding increase in optical density showed that degradation of the DNA had occurred (Fig. 3).

Effect of u.v. irradiation on transforming DNA

Figure 4 shows the effect of u.v. irradiation on inactivation of transforming principle in *Aspergillus niger* (v35). There was a decrease in the transformation activity with successive increase in the time period of irradiation.

DISCUSSION

The results show that transformation process occurs in *Aspergillus niger*. The frequency of transformation is low as compared to the bacterial systems where transformation is known to occur. Transformation to prototrophy in bacteria has been observed by Spizizen (1958), Gwinn & Thorne (1964) and Leonard, Corley & Cole (1966). Shamoian *et al.* (1961) and Shockley & Tatum (1962) attempted the transformation of auxotrophic strains of *Neurospora crassa* to wild type, and the latter authors were of the opinion that the results provided suggestive but not convincing evidence of transformation in this strain. The present experiments show that there is

Fig. 3

Fig. 3. Heat inactivation of transforming DNA. (Donor, v35; recipient, 45 *arg*⁻.)

Fig. 4

Fig. 4. Ultraviolet inactivation of transforming DNA. (Donor, v35; recipient, 45 *arg*⁻.)

a similarity with the findings of Hotchkiss (1951) and Marmur & Fluke (1955) in the response to concentration changes of donor DNA. The effect of heat, DNase and u.v. irradiation on donor DNA resulting in the inactivation of the transforming principle in *A. niger* is more or less similar to that reported by Lerman & Tolmach (1959) for *Pneumococcus*. The transformants scored in the present investigation were found to be stable.

The lower frequency of transformation reported here, as compared to bacterial system, might be attributed to a number of factors. The growth and multiplication of bacteria differ widely from those of the fungi and the environmental factors required for transformation are perhaps not similar to those applicable in the case of bacteria. Nothing is known as yet about the nature of competence in fungi, which depends on factors like structure of the recipient cell surface, production of DNase by the cell or the requirement of a specific nutrient to make the cells competent. Lower frequency of transformation could also be attributed to the method of extraction of DNA since crushing the cells in the presence of alumina possibly breaks the DNA into small

fragments thus making it biologically less active. Transformation which occurred in the present experiments could be deduced from two observations: (1) no reversions occurred in the controls, i.e. strains which were not treated with the donor DNA, or, were treated with their own DNA; (2) the transformation frequency diminished or totally disappeared when the transforming principle (the donor DNA) was subjected to treatment with heat, DNase or u.v. irradiation.

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