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Difference between transformed and spontaneous revertant strains of Neurospora crassa

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Abstract

Difference between transformed and spontaneous revertant strains of *Neurospora crassa*

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During the port few years several papers hove been published concerning the genetic transformation of N. crassa. (Mirhro and Tatum(1973) Proc. Nat. Acad. Sci. USA 70: 3875; Mirhro, Szabó and Tatum (1973) in, The Role of RNA in Reproduction and Development, Ed. M.C. Niu and S.J. Segal, North-Holland Pub,. Co., Amsterdam, p. 259; Schablik et al. Acad. Sci. Hung. [in press]) The results obtained so far indicate that the efficiency of the transformation process is low and that transformants are relatively unstable as compared to spontaneous revertants. In addition, Mishra, Szabó and Tatum op. cit. observed that the growth of some DNA-induced transformants was slow. Further observations are reported here on the growth and stability of transformed (inl → inl$^+$) derivatives of inl (89601), ra (R2357) a strain(R2506-5-101) compared to that of spontaneous revertants. The origins of the strains used in this study were described previously (Mishra and Tatum (1973) op cit).

TABLE I

Comparison of growth rates$^+$ of spontaneous revertants, transformants, and inl on minimal and inositol supplemented medium

strain	medium: minimal	medium: minimal + inositol
inl(R2506-5-101)		4.68
spontaneous revertants (5 strains)	average: 4.36 ±1.28	overage: 4.52 ± 1.76
transformants (7 strains)	average: 3.08 ±0.71	average: 3.14 ±0.91

$^+$Dry weight at 48 hr/dry weight at 24 hr.

Several different tronrformed strains and spontaneous revertontr were grown on Vogel's minimal medium to determine their vegetative growth rates. Eight milliliters of medium in twenty-five milliliter Erlenmeyer flasks were inoculated with 4x 10^4 hyphal fragments/ml and incubated at 27° C at 240 rpm on a rotary shaker. At 24 and 48 hours, the resulting mycelia was harvested, washed with distilled water and dried at 105° C for 24 hours. Growth rate was estimated as the ratio of the dry weight after 24 hours growth to the dry weight after 48 hours growth. The average growth rate of the transformed strains was significantly lower than that of the spontaneous revertants or the R2506-5-101 progenitor strain (Table I). This lower growth rate might be explained by the possibility that the transforming DNA may integrate into a number of chromosome sites, thereby increasing the probability of mutations and chromosomal aberrations during subsequent vegetative nuclear multiplications. The low growth rate of the tronrformed strains was found to be stable when the strains were propagated continuously on minimal medium.

TABLE II

Tetrad types from crosses of two inl$^+$ transformants and one spontaneous revertant with inl

cross	number of asci: 4:4	6:2	2:6	5:3	0:8	Total
transformant #5 x inl	33	1	2	2	32	70
transformant #6 x inl	19	1	0	0	14	34
(Tspontaneousrevertant K/2 x inl	29	0	0	0	0	29
wild type (RL#-8) x inl	29	0	0	0	0	29

In the first 3 crosses inl = strain 89601-5-5 A; in the last inl = strain R2506-5-101 (which contains inl allele 89601)

The stability of the DNA-induced inl$^+$ character was also studied during the sexual phase of growth. Two transformants (No-. 5 and No. 6), one spontaneous revertant, and one standard wild type strain the RL3-8 A were crossed with inl$^-$ strains (89601 and R2506). For tetrad analysis n-d-day-old perithecia were dissected and ascospores were isolated from complete asci. The morphology and inositol-requirement of the colonies grown from the tetrads were studied.

In the tronrformed strains No. 5 and No. 6 a large number of asci containing only inositol-requiring ascospores were found, probably because there strains are hetercaryotic for inl (Table II). Besides the regular Mendelian (4 inl$^+$: 4 inl) tetrads some non-Mendelian ones of the types 2 inl$^+$: 6 inl, 6 inl$^+$: 2 inl, 5 inl$^+$: 3 inl were also obtained. There non-Mendelian tetrads may be the result of gene conversion or the resolution of chromosome aberrations, both of which could result from the integration of the transforming DNA with the recipient genome.

The increased number of gene conversion. found in our earlier investigation (Schablik et al. (1977) Neurospora Newsl. 24:4) was not discovered probably become of the limited number of asci - containing inl$^+$ ascospores - ascertained. - Institutes of Biology and Biochemistry, University Medical School, H-4012 Debrecen, Hungary.