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Characterization in inl⁺ transformants of Neurospora crassa obtained with a recombinant cosmid-pool

Abstract
Characterization in inl⁺ transformants of Neurospora crassa obtained with a recombinant cosmid-pool

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Table II
Transformation of various derived strains

| OSU Strain Number* | Outcrossed with strain (FCSC no.) | Mean viability qa-2+ Transformant/µg DNA** |
|-------------------|-----------------------------------|------------------------------------------|
| 249 (= standard strain) | 29 (11) | 105 (79) |
| 179A W.T. Lein 7A (847) | 24 (0.5) | 203 (41) |
| 181A W.T. Costa Rica (651) | 21 (7) | 41 (26) |
| 187A W.T. Puerto Rico (429) | 42 (3) | 18 (5) |
| 191A W.T. Tatum 14 | 14 (0.5) | 116 (8) |
| 283 | nit-2 (982) | 39 (1) | 49 (27) |
| 284 | nit-2 (982) | 47 (5) | 19 (2) |
| 288 | nit-2 (982) | 33 (14) | 35 (5) |
| 289 | nit-2 (982) | 12 (1.5) | 75 (15) |
| 290 | nit-2 (982) | 21 (9) | 94 (4) |
| 305 | os-1 | 33 (16) | 15 (3) |
| 197A W.T. Costa Rica 74-0823-1 (988) | 33 -- | 15 -- |
| 268A cys-3 (1090) | 41 -- | 37 -- |

*All strains are inl;qa-2;aro-9, except that #305 (os-l;qa-2;aro-9), which was obtained from R.L. Metzenberg, lacks inl. Strains #283 also carry the nit-2 mutation and #268 carries cys-3.

**Mean value, corrected to 100% viability, for 2 independent transformations is given, except that 197A and 268A values are for a single experiment.

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Characterization of inl+ transformants of Neurospora crassa obtained with a recombinant cosmid-pool.

An N. crassa gene library was constructed in a BHB 3030 yeast cosmid vector (Feher 1984 Neurospora News1. 31: 32). Each E. coli clone of the library contains recombinant DNA molecules i.e. fragments of Neurospora DNA inserted into the BamHI site of the cosmid vector. DNA was prepared from all 5000 clones of the library (a recombinant cosmid-pool) and it was purified further on a CsCl-ethidium bromide gradient (Maniatis et al. 1982: Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). 20 µg of the cosmid-pool DNA was used to transform protoplasts (Schablik et al.1983 Neurospora News1. 30: 17) of an inl+,N. crassa strain (R2506), according to the procedure of Case (1982 in: Genetic Engineering of Microorganisms for Chemicals, eds. Hollaender et al. pp. 87-100 Plenum, New York) with slight modifications. Two inl+ transformants (T1 and T3) were obtained. They were back-crossed to an inl strain and several inl+ ascospores from the F1 progeny were selected for further genetic and biochemical studies.

The inl+ phenotype proved to be inherited as a chromosomal gene on linkage group V. The map distance from the neighbouring al-3 locus was 0.58% and 2.46% for T1 and T3, respectively. This value in the wild type strain was 1.16%. These results convincingly show that transformants have inl+ genes closely linked to al-3. One of the transformants, T1, exhibited mitotic and meiotic instability.

Cross-immunoelectrophoresis of the purified gene product (MIPS) showed that the transformants synthesize both the "wild type" enzyme and the inactive enzyme-protein. The specific activity (enzymatic activity / µg antigen) of the enzyme purified from the transformants proved to be about 50% lower than that of the wild type enzyme. Physiological regulation of MIPS synthesis -"inositol repression" - was same in the transformants as in the control.
These results suggest that an integration event resulted in a tandem arrangement of \textit{inl} sequences leaving the expression of both \textit{inl} and \textit{inl} genes intact.

In Southern hybridization experiments, bands characteristic for covalently closed circular plasmids were detected in the DNA isolated from the F1 progeny of the transformants using the vector as a hybridization probe. (The principle of the detection was that the supercoiled plasmid DNA migrates faster than uncleaved chromosomal DNA in an agarose gel-electrophoresis.) The copy-number of the transforming cosmid sequences was estimated to be 20-25/gene right after transformation and 1-2/gene after two months of vegetative propagation on agar slants with periodic transfers. Eleven recombinant plasmids (pNCs) with different restriction patterns were recovered from the transformants by means of E. coli transformation. Some of these plasmids proved to be rearranged in \textit{N. crassa}, e.g. deletions within the vector sequences could be detected. None of the pNCs transformed \textit{Neurospora} to inositol prototrophy. There are a number of explanations for the presence of different plasmids in the F1 progeny of integrative-type transformants: (1) The plasmids were maintained during meiosis by autonomous replication. (2) They are the result of excision from the chromosome. (3) chromosomal DNA fragments carrying vector sequences were taken up and circularized by \textit{E. coli}.

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1Kiss, A., 1A, Zsindely, The synthesis of myo-inositol-1-phosphate synthase (MIPS, E.C.5.5.1.4.) in wild-type \textit{Neurospora crassa} strains is almost completely repressed by inositol at a concentration of 50 µg/ml (Zsindely et al., 1983 Biochim. Biophys. Acta 741:273).

Effect of various inhibitors of the production of myo-inositol-1-phosphate synthase in \textit{Neurospora crassa} wild type strain.

We studied whether the enzyme was derepressed after removing inositol from the medium. Wild-type \textit{Neurospora crassa} strain RL-3-8A was grown at 27° C for 22 h in Vogel's culture medium containing 50 µg/ml inositol. Following harvest, the mycelium was washed, suspended in Vogel's minimal medium and growth was continued for 22 h during which samples were taken at various times. Enzyme activity and amount of enzyme protein were determined in the 100,000 g supernatant after disintegration of the mycelium (Table I). Enzyme activity was determined according to Barnett et al. (1970, Biochem. J. 119: 183), as described earlier (Zsindely et al., 1977, Acta Biol. Acad. Sci. Hung. 28:281). One unit of activity is 1 nmol Pi released during 1 h incubation. The amount of protein reacting with monovalent immune sera produced against highly purified enzyme was determined by rocket immunoelectrophoresis according to Laurel.1 (1966, Anal. Biochem. 15: 45) in a 1% agarose gel containing 1% immune serum.

Table I shows that MIPS becomes derepressed after removing inositol from the culture medium. Four h later the enzyme activity and the antigen content become similar to those measured in the crude extracts of the wild-type strain cultivated without inositol. No further change in enzyme activity or antigen content was observed up to 22 h of cultivation.