Detection and identification of myo-inositol-1-phosphate synthase and its assumed defective variant in different Neurospora crassa strains by immunological methods

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Abstract
Detection and identification of myo-inositol-1-phosphate synthase and its assumed defective variant in different Neurospora crassa strains by immunological methods

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Differences between transformed and spontaneous revertant strains of Neurospora crassa.

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 (1973) observed that the growth of some DNA-induced transformants was slow. Further observations are reported here on the growth and stability of transformed (inl) derivatives of inl (89601), e.g., (R2506-5-101) compared to that of spontaneous revertants.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparison of growth rates of spontaneous revertants, transformants, and inl on minimal and inositol supplemented medium</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>strain</th>
<th>minimal medium</th>
<th>minimal + inositol</th>
</tr>
</thead>
<tbody>
<tr>
<td>inl (R2506-5-101)</td>
<td>4.68</td>
<td></td>
</tr>
<tr>
<td>spontaneous revertants (5 strains)</td>
<td>average: 4.36 ± 1.28</td>
<td>average: 4.52 ± 1.76</td>
</tr>
<tr>
<td>transformants (7 strains)</td>
<td>average: 3.08 ± 0.71</td>
<td>average: 3.14 ± 0.91</td>
</tr>
</tbody>
</table>

*Dry weight at 48 hr/dry weight at 24 hr.*

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrad types from crosses of two inl transformants and one spontaneous revertant with inl</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>cross</th>
<th>number of ascii</th>
</tr>
</thead>
<tbody>
<tr>
<td>inl x K-2 x inl</td>
<td>4:4 6:2 2:6 5:3 0:8 Total</td>
</tr>
<tr>
<td>33 1 2 2 32 70</td>
<td></td>
</tr>
</tbody>
</table>

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

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 (Szabó et al. 1977) Neurospora News. 24: 4) was not observed. It is possible to become the limited number of inl-containing inl oscospores - ascertained, several non-Mendelian ones of the type 2 inl: 6 inl, 3 inl: 5 inl 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.

In the transformed strain No. 5 and No. 6 a large number of ascii containing only inl oscospores were found, probably because there strains are heteroecic for inl (Table II). Besides the regular Mendelian (4 inl: 4 inl) tetrads some non-Mendelian ones of the type 2 inl: 6 inl, 3 inl: 5 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.

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The identification of assumed "defective form" of MIPS was attempted with immune sera produced against wild type enzyme. Mice were immunized interperitoneally with MIPS according to Tung et al. (1976, J. Immunol. 116: 676-681) with complete Freund adjuvant. After three weeks the mice were given a second injection and they were treated with Ehrlich ascites tumor cells. Two weeks later the peritoneal exudates were removed. After centrifugation the clear exudates were used as "antisera" for antigen analysis. In immunodiffusion experiments performed according to Ouchterlony in agarose gels (Figure 1), MIPS resulted in a single precipitation band and its intensity was proportional to enzyme concentration (10, 5 and 2.5 μg protein). A similar but much weaker precipitation band was obtained with the protein fraction isolated from inl.

To define which electrophoretic species is responsible for the immune response a procedure was developed to accomplish the immune reaction with antisera directly in the gel tube immediately after electrophoresis. A similar method was described by Makonawkeyoon and Haque (1970, Anal. Biochem., 36: 422-427). In the "immunodisc" electrophoretic technique acrylamide was polymerized as hollow cylinders by placing thin plexiglass rods in the middle of tubes which were immediately removed after the electrophoresis. Then one of the parallel samplers was directly stained while the other one was used for antigen analysis, i.e., the lumen was filled with antisera diluted in saline. After immunoprecipitation other proteins that were not precipitated were removed by efficient washing and the gel also stained. The comparison of purified MIPS and the fraction isolated from the inl mutant (Figure 2) show that in contrast to the nearly homogeneous MIPS from the wild type, the protein fraction from the mutant consisted of several components, but only one of them had a somewhat lower mobility than that of the wild type enzyme resulted in a definite immune response. In addition, a weak reaction was given by another component of a much lower mobility.

Investigations were also performed with a transformant strain, obtained by treatment with DNA of wild type (allo-DNA) and with spontaneous extracts of both strains. Positive immune responses were detected in strains and some properties of them were compared to MIPS from the wild type. A well pronounced MIPS activity was measured in by "immunodisc" electrophoresis. The enzymes were isolated from both strains and some properties of them were compared to MIPS from the wild type. Their specific activities were lower than those of wild type enzyme.

![Figure 1. Immunodiffusion of MIPS and the protein fraction isolated from inl (89601). Experiment was carried out in 0.6% agarose gel, 0.1 ml antiserum III, staining with Amido-black 10B. Wells 1, 3, 5 contained MIPS from the wild type, 10, 5 and 2.5 μg protein respectively. Wells 2, 4, 6 contained the protein fraction from inl 10, 5 and 2.5 μg protein respectively.](image1)

![Figure 2. Immunodisc electrophoresis of MIPS and of the protein fraction isolated from inl (89601). Antiserum was diluted with saline in 1 to 2 ratio. 1 and 2: MIPS from wild type (300 μg protein), 3, 4, and 5: the protein fraction from inl (100 μg protein). 1 and 3 were stained directly with Coomassie brilliant blue, 2, 4, and 5 were stained after immunoprecipitation.](image2)

![Figure 3. Immunodisc electrophoresis of MIPS isolated from different strains of N. crassa. The antiserum was diluted with saline in 1 to 2 ratio. 1 and 2: MIPS from wild type (300 μg protein), 3 and 4: MIPS from transformant (60 μg protein), 5 and 6: MIPS from spontaneous bock mutant (100 μg protein). 1, 3, and 5 were stained directly after run. 2, 4, and 6 were stained after immunoreaction.](image3)

The gel electrophoretic patterns were very similar to that of the protein fraction of inl mutant, i.e., both products contained several proteins (Figure 3), but proteins that had nearly identical electrophoretic mobilities as that of MIPS from wild type gave a positive immune response. In addition, both protein fractions contained an additional immunological reacting component that ran faster than the wild type enzyme.

These results definitely support the idea that an inactive, defective protein homologous with MIPS of wild type is synthesized in the inositol requiring mutant allele 89601 and that the preparative procedure applied is suitable for its concentration and isolation. An enzyme species produced by the transformed strain is probably identical to that of the donor DNA strain, and to an enzyme species produced by the spontaneous revertant. (This work was supported by grants of the Hungarian Ministry of Health and the Hungarian Academy of Sciences.)

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