

Kiss, A., M. Schablik*, A. Zsindely

and G. Szabo*

Characterization of a slow growing

inl⁺ revertant Neurospora crassa

strain.

measured in crude extracts of wild type (Kiss et al. 1984, Neurospora Newsl. 31:25-26; 1985, Neurospora Newsl. 32:9-10). The spontaneous reversion rate of inl mutant strain 89601 is 10^{-8} - 10^{-9} (N.H. Giles, 1956, Cold Spring Harbor Symposia on Quantitative Biology XVI:283-313).

In this study, we characterized the MIPS activity and antigen content (CRM) of an inl⁺ (483-7) strain showing slow growth on minimal medium. This strain was obtained as a spontaneous partial revertant from the inl strain no. 89601. We wanted to find out whether the slow growth was due to the small quantity of MIPS or to low enzyme activity.

The strains were grown at 27°C in submerged culture for 22 h in Vogel's medium N containing 3 ug/ml inositol. The 100,000 x g supernatants of crude extracts (Zsindely et al. 1977, Acta Biol. Acad. Sci. Hung. 28:281-290) were used to determine enzyme activity by the method of Barnett et al. (1970, Biochem J. 119:183-186), antigen content according to Laurell (1966, Anal. Biochem. 15:45-49) and protein content by the Biuret method (Zsindely et al. 1983, Biochem. Biophys. Acta 741:273-278).

Table I shows the change in antigen content and the MIPS activity of RL-3-8A, homo-caryotic (f1) inl⁺ (483-7) and inl (89601) strains at various inositol concentrations in the medium. When growth is carried out in the presence of 3 ug/ml inositol, the antigen content is ten times higher than that measured in the wild-type strain, and 4 times higher than that in the inl strain under similar conditions. With increasing inositol concentration, the antigen content becomes lower in each of the three strains. In the case of the wild type strain, even at an inositol concentration of 3 ug/ml and 6 ug/ml the inhibition is significant, i.e. 36% and 60%, respectively. Strain 483-7 seems to be less sensitive up to 6 ug/ml inositol. Specific activity of MIPS in strain 483-7 seems to be much lower than that of the wild type enzyme. Thus it seems that it is the partially inactive cross reacting (CRM) in high quantity which enables this strain to grow slowly on minimal medium. The enhanced production of CRM in strain 483-7 might be the result of a regulatory gene mutation. To test this hypothesis, strain 483-7 was crossed to an inl⁺ strain and ordered tetrads were analysed for MIPS activity and antigen content.

An inositol requiring Neurospora crassa mutant (89601) produces defective enzyme of the same molecular weight as that of myo-inositol-1-phosphate enzyme (MIPS, E.C. 5.5.1.4.) isolated from the wild-type RL-3-8A strain. The defective enzyme shows cross reaction with anti-MIPS serum (Zsindely et al. 1979, Acta Biol. Acad. Sci. Hung. 30:141-149). The defective enzyme has residual activity which, in osmotically stabilized medium, may reach 80% of the enzyme activity

Table II presents the enzyme activity as well as the antigen content of four members of an ordered tetrad. The strains were grown at 27°C for 22 h in Vogel's medium containing 3 ug/ml inositol. The enzyme activity and antigen content were determined in the 100,000 x g supernatant. Three members of the tetrad appeared to be of inl⁺ phenotype, and one was inl⁻. Two inl⁺ members (spores 2 and 4) contain as much antigen as the wild-type strain. The inl⁻ member (spore 6) is a derepressed antigen producer, but does not grow on minimal medium. The slowly growing member (spore 8) is an antigen overproducer inl⁻ phenotype. On the basis of the specific activity, the tetrad contains two inl⁺ and two inl⁻ strains. These data indicate that strain 483-7 produces a high quantity of CRM.

Since the crossing of strain 483-7 to the inl⁺ strain resulted in 6 inl⁺ and 2 inl⁻ tetrads (Table II), we suppose that the overproduction of the antigen i.e. the suppression of the inositolless phenotype, is due to mutation of a regulatory gene unlinked to the inositol locus (present in spore 8 and absent in spore 6). - - - Departments of Biochemistry and *Biology, University Medical School of Debrecen, H-4012 Hungary.

Table I
Effect of inositol upon the production of MIPS or CRM in various strains of N. crassa

RL-3-8A				483-7		89601	
Inositol ug/ml medium	Enzyme activity U/mg protein	Antigen content ug/mg protein	Specific activity U/ug antigen	Enzyme activity U/mg protein	CRM content ug/mg protein	Specific activity U/ug antigen	CRM content ug/mg protein
0.0	88	59	1.49	n.d.	n.d.	n.d.	n.d.
2.0	81	52	1.56	n.d.	n.d.	-	153
3.0	73	50	1.46	23.3	487	0.048	122
6.0	58	38	1.52	12.3	418	0.029	60
12.0	23	15	1.53	+/-	70	-	37
25.0	20	13	1.54	n.d.	n.d.	-	11
50.0	15	11	1.36	+/-	43	-	11

CRM : cross reacting material
n.d. : not determined
+/- : in traces, is not measurable

Table II
Ordered tetrad from a cross between inl⁺ and strains 483-7 (inl⁺; su⁺) and 1985-4 (al-3, inl⁺; su)

Spores	Phenotype	Enzyme activity U/mg protein	CRM content ug/mg protein	Specific activity U/ug CRM
2	al-3, <u>inl⁺</u>	37.1	23.5	1.6
4	al-3, <u>inl⁺</u>	48.1	28.7	1.7
6	al-3 ⁺ , <u>inl</u>	11.0	227.3	0.05
8	al-3 ⁺ , <u>inl⁺</u>	18.8	632.6	0.03

CRM : cross reacting material