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Abstract

Four mitochondrial loci in *Podospora anserina*

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In a previous paper we described five mitochondrial mutations in Podospora anserina and showed by genetical analysis that they belong to three loci, two of which are closely linked (Belcour and Begel 1977 Mol. gen. Genet. 153: 11-21). Here we describe a new mitochondrial mutation, [mit b] belonging to another locus.

Table 1 giver schematically the phenotypic properties of the [mit b] mutant compared to that of wild type strain and the other known mitochondrial mutants. The [mitrb]ns are characterized by slow growth, complete lack of cytochrome aa3, female sterility and cyanide-insensitive respiration. Their behaviour on media complemented with a tetrazolium salt (inhibitor of the cytochromic electron transport chain) and with salicylhydroxamic acid (SHAM: inhibitor of an "alternative" respiratory pathway in higher plants and other organisms) seems to confirm the inefficienc, of the normal cytochromic respiratory pathway and the activity of an alternative cyanide insensitive and SHAM sensitive pathway. All the observations presented here have been mode on whole mycelium; further study using purified mitochondria is needed.

Table 2 summarizes the results of all the crosses to date (mutant x wild type and mutant x mutant) made in standard conditions. In crosses mutant x wild type, the low recovery rate (transmission rate) of each mutation is the result of a strong selection pressure favoring wild type mitochondria in a mixed population (as shown previously). In crosses involving [mit b] we obtained only in two cases strains showing exactly the same properties as the [mit b] parental strain. However, about two percent of the progeny showed a phenotype clearly different from that of the wild type parental strain. These strains have received at least some mitochondria from the [mit b] parent. The properties of these strains will be briefly discussed below.

In all mutant x mutant crosses listed in Table 2 we obtained asci displaying wild phenotype for germination. A sample of progeny from this cross was further studied for other phenotypic properties and genetic stability. With a few exceptions (mainly in crosses involving [mit b]: see below) these strains displayed wild type properties and were found to be pure. Wild type recombinants have thus been obtained in all the crosses, indicating that the mutations studied belong to four distinct loci. In addition it can be noted that: 1) the frequency of wild type recombinant strains can be higher than 80% and 2) in no crosses have reciprocal double mutant recombinant strains been detected. These two facts could be explained by the selection pressure strongly favoring wild type genotypes as shown by crosses mutant x wild type.

From the quantitative data given in Table 2 recombination rate between two mutations can be estimated taking into account on the one hand the frequency of wild type recombinant progenies obtained in crosses mutant x mutant, and on the other hand the relative selective values of each parental genotype with respect to the wild type genotype (deduced from the results of crosses mutant x wild type). The values of this parameter R measuring the genetical distances between mutations ore given in Table 2. Although the distances calculated do not show additivity they are consistent with a unique order of the map, (except for the relative order of [spg 1] and [spg 2]). Furthermore, tight linkage between [spg 1] and [spg 2, is confirmed and a looser linkage between [cap^r 1] and [mit b] can be assumed.

A few percent of the strains from crosses involving the mutation [mit b] showed phenotypic properties different from that of both parental strains (and from that of wild type strains). These progeny came either from wild type or from abnormal germinating asci and either did or did not display norm., growth in the first centimeters of growth. But their common property is to stop growth either soon

TABLE 1

Phenotype of the mitochondriol mutants

	(a) Growth rate mm/d	(b) female fertility	(c) germination phenotype	(d) cytochrome content c/aa$_3$	(e) cyanide resistant respiration expo.	(e) stat.	(f) CAP resistance 3mg/ml	(g) SHAM resistance 0.2mg/ml	(h) TETRA 0.5mg/ml
w.t.	5.2	+++	+++	2.4 to 3.6	S	S	S	R	Pink
[spg1]	4.5	++	+	5.5 to 9.0	S	S	S	R	Pink
[spg2]	4.7	++	+	5.5 to 9.0	S	S	S	R	Pink
[cap^{r}1]	4.7	++	++	30	R	S	R	R	Pink
[mit b]	2.4	-	?	∞	R	R	R	S	White

Footnotes to Table 1:

The phenotypic properties of the mitochondriol mutants, with the exception of [mit b] have in port already been described by Belcour and &gel (1977). w.t. stands for wild type s strain. [spg1] stands for any of the mutation formerly named (64), (89) or (119) that could not be separated by recombination and display the same phenotypic properties: [spg2] stands for the mutation formerly named (561). (a): measured as the linear elongation of mycelium on solid medium at 27° C; (b): determined by the number of perithecia in crosses ♀ mutant x ♂ w.t. in standard conditions; (c): determined by the diameter of the mycelia from germinated ascospores after 48 hours incubation a, 27° C; (d): measured at liquid nitrogen temperature on whole mycelium pastes reduced by dithionite. The number are the ratios of the height of the peaks of cytochrome c and cytochrome aa3 above a definite baseline (see: Belcour and Begel, 1977); (3): measured on whole mycelium in a Gilson oxygraph with a Clark oxygen electrode. S = less than 30% of oxygen consumption is resistant to 5mM KCN, R = more than 60% of oxygen consumption is resistant to 5mM KCN. (expo. and stat. stand for exponential and stationary phases of growth,; (f): chloramphenicol (CAP) is added in synthetic solid medium. mit b] shows a good elongation rate on CAP-containing medium but develops a very low density of hyphae; (g): Salicylhydroxamic acid (SHAM) is added to synthetic medium neutralized at pH 7.0; (h): 2,3,5-triphyltetrazolium chloride (TETRA) is added to synthetic medium neutralized at pH 7.0. Mycelia grown on permeable cellophane discs laid on synthetic solid medium were transferred to TETRA-containing medium and incubated at 27° C. Coloration of mycelia was examined after 2 and 4 days of incubation. Reduction of TETRA by the electron flow through the cytochrome pathway changes it. coloration from white to pink.

after germination or after a few centimeters of growth. Development of a dark pigmentation at the edge of the culture parallels stoppage of mycelial growth. These lethal strains seem to result from mixing of [mit b] mitochondria with mitochondria of any other genotype. A few progeny from [spg 1] x [spg 2] crosses also displayed a very similar phenotype. The study of such strains will be presented elsewhere. We assume that recombination between [mit b] and any other type of mitochondria and between [spg 1] x [spg 2] mitochondria give rise to some suppressive and lethal genotype.

TABLE 2

Genetical studies of mitochondrial mutants

CROSS				PROGENY			R (a)
Parent A		Parent B	no of asci scored	% A	% B	% w.t. recombinant	recombination rate
[spg 1]	x	w.t.	2,000	1	99	-	-
[spg 2]	x	w.t.	1,000	1	99	-	-
[cap^{r}1]	x	w.t.	3,000	9	91	-	-
[mit b]	x	w.t.	1,600	2*	98	-	-
[spg 1]	x	[spg 2]	15,000	-	98#	2	0.04
[spg 1]	x	[cap^{r}1]	2,000	2	64	34	4.9
[spg 2]	x	[cap^{r}1]	1,800	6	46	47	8.1
[spg 1]	x	[mit b]	200	-	12*#	88	18.0
[spg 2]	x	[mit b]	1,400	6	7*	87	17.0
[cap^{r}1]	x	[mit b]		90	1*	9	1.1

All the crosses performed in the standard conditions defined by Belcour and Begel (1977). The phenotype of germination of asci were recorded after 48 hr of incubation at 27°C. A sample of strains of each phenotypic category was used for a more complete phenotypic study (growth rate, cytochrome spectra, drug sensitivity etc..) and for genetical studies (stability in further generations).

(a) R is a parameter estimating the genetical distance between the mutations, taking into account both the frequency of wild type recombinant strains in mutant x mutant crosses and the transmission rate of each mutant in crosses mutant x w.t.

*Most of there strains do not display the complete ret of phenotypic properties of the parental [mit b] strains and hove received at least some mitochondrial information from the [mit b] parent.

#The two parental phenotypes hove not been distinguished.

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