Fungal Genetics Reports

Volume 45 Article 9

UV light induced accumulation of variability in a diploid strain of Aspergillus nidulans

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Recommended Citation

Marini, A., S. PIMPINELLI, N. MATMATI, N. BABUDRI, and G. MORPURGO (1998) "UV light induced accumulation of variability in a diploid strain of Aspergillus nidulans," *Fungal Genetics Reports*: Vol. 45, Article 9. https://doi.org/10.4148/1941-4765.1256

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Abstract

The accumulated variability in asexual species was evaluated in *Aspergillus nidulans* diploid cells after repeated cycles of UV irradiation. The results show that diploid cells can accumulate a very high genetic variability in the heterozygous condition as previously shown with the base analog 6-N-hydroxylaminopurine (HAP).

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UV light induced accumulation of variability in a diploid strain of *Aspergillus nidulans*

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The accumulated variability in asexual species was evaluated in *Aspergillus nidulans* diploid cells after repeated cycles of UV irradiation. The results show that diploid cells can accumulate a very high genetic variability in the heterozygous condition as previously shown with the base analog 6-N-hydroxylaminopurine (HAP).

In a previous paper (Pimpinelli *et al.* 1997 Curr. Genet. **32**: 331-336) we have shown that an enormous amount of genetic variability is accumulated in *Aspergillus nidulans* diploid, asexually reproducing, cells after repeated treatments with the base analog HAP which causes only base substitutions. We have estimated that after twelve HAP treatments the diploid conidia are likely to differ from each other for several mutations without loss of conidial viability. Since HAP causes only base substitutions, we decided to study also the effects of a wide spectrum mutagen, such as UV light, in the diploid strain *A513/35 pabaA1*, which has been constructed with the haploid strain *A513* and the strain *35 pabaA1* (Table 1).

Six plates (complete medium) were inoculated with 10^6 conidia/plate and immediately irradiated for 125 sec (surviving fraction 21%). After two days of incubation at 37°C, conidia were collected and used for the next mutagenesis cycle as well as for determination of the accumulated variability by the 8-azaguanine resistance test (8-AZA-R test) and by estimation of auxotrophic mutants. The 8-AZA-R test used in this work was a modification of the one used with haploid strains (Morpurgo 1962 Sci. Rep. Ist. Sup. Sanità **2**: 9-12) and allows the detection of heterozygous ($aza^R/+$) diploid cells. Diploid conidia were plated on minimal medium plus nutritional requirements except adenine, which competes with 8-AZA, and allowed to grow for 12 h.
Then a second layer of minimal medium plus 8-AZA (final concentration in the medium: 5 mg/ml) was added and the plates were incubated for 6-7 days. At that time aza^R sectors, arising from $aza^R/+$ colonies via mitotic segregation, were well grown and readily detectable.

Auxotrophic mutant frequency was determined as described in Pimpinelli *et al.* (Curr. Genet **32**: 331-336) after haploidization by Benomyl. It was possible to obtain a sufficiently high number of sectors up to 9th cycle; thereafter the percentage of sectoring colonies was very low (Table 2) probably because of the high number of lethal mutations and especially chromosomal aberrations. The frequency of $aza^R/+$ cells is reported in Table 3. In the first cycle the frequency was 0.6×10^{-3}, a value very close to the one obtained in the haploid strain *35pabaA1* (0.45×10^{-3}). In the diploid, the mutation frequency increased in the subsequent cycles up to 9.0×10^{-3} (9th cycle). In the haploid strain *35 pabaA1* the mutation frequency reached a plateau at the 5th cycle (3.0×10^{-3}).

This lower mutation frequency can be explained by selection against aza^R mutants. Since selection could have occurred also against $aza^R/+$ heterozygous, we have done reconstruction experiments where +/+ and $aza^R/+$ cells were mixed and their viability with or without irradiation were examined . These experiments excluded the loss of $aza^R/+$ mutants during UV irradiation cycles. Conidia of ten $aza^R/+$ clones obtained independently were mixed with +/+ conidia. The percentage of the $aza^R/+$ conidia was 39%. 2×10^5 conidia were plated on CM and part of the dishes were irradiated 125 sec. The percentage of $aza^R/+$ conidia were tested on a sample of the conidia grown on the dishes with and without irradiation. Data are reported in Table 4 in which no significant difference in viability of the $aza^R/+$ conidia was detected.

The accumulation of variability was also evaluated by the frequency of the diploid heterozygous for

auxotrophic mutations. The data reported in Table 5 indicate that also this class of mutants increased between the 1st and 6th cycle. Ten auxotrophic mutants were analyzed and they have shown the following requirements: riboflavin (4), lysine (2), arginine or proline (1), inositol (1), nicotinic acid (1) and nicotinic acid + thiamin (1). On the basis of these data we can conclude that the accumulated variability is very high not only after repeated cycles with a base analog (HAP) (Pimpinelli et al. 1997 Curr. Genet. 32: 331-336), but also with a broad range mutagen such as UV light. However with UV light the frequency of the sectoring colonies was reduced to about 50% already at the 5th cycle while it was 44% at the 12th cycle with HAP; moreover after 9 cycles of UV irradiation the colonies grew and conidiated poorly, probably because of UV-induced chromosomal aberrations. Appearance of Benomyl resistant colonies cannot account for the reduced sectoring because all the colonies transferred on medium with Benomyl turned out to be sensitive to the drug.

Table 1. Genotype and origin of strains

Strain	Genotype	Origin
35 pabaA1	pabaA1, yA2	Our collection
A513	adE20; acrA1; actA; pyroA3; facA303; lacA, sB3; chaA1	FGSC[1]

[1] Fungal Genetics Stock Center, Kansas City, USA

Table 2. Benomyl-induced sectoring in the *A513/35pabaA1* strain after UV treatment cycles

UV cycle N°	N° of colonies tested	% of sectoring colonies
0	2,889	92
1	252	90
2	377	85
3	209	76
4	416	69
5	458	48
6	443	44
7	338	41
8	327	38
9	324	18
10	133	9

Table 3. UV-induced aza^R/+ mutants in the diploid strain *A513/35 pabaA1* (irradiation time: 125 secs)[1]

UV cycle N°	N° of viable conidia plated	N° of aza^R/+ mutants	Mutation frequency ($x10^{-3}$)
1	9,397	6	0.6
4	8,458	27	3.2
6	9,870	49	4.9
9	5,600	52	9.3
12	8,176	74	9.0

[1] The spontaneous mutation frequency was $0.9x10^{-6}$

Table 4. Results of reconstruction experiment

UV dose	Colonies tested	$aza^R/+$ conidia	% $aza^R/+$
0"	160	62	39
125"	160	54	34

Table 5. UV-induced auxotrophic mutants in the diploid strain *A513/35pabaA1* (irradiation time: 125 secs)[1]

UV cycle N°	N° of haploid sectors tested	# of auxotrophic mutants	Mutation frequency ($x10^{-2}$)
1	1,413	12	1.0
4	394	13	3.3
6	501	24	4.8
9	122	6	4.9

[1] The spontaneous mutation frequency was below $1x10^{-3}$