Asexual Recombination in a *uvsH* Mutant of *Aspergillus nidulans*

SOUZA-JÚNIOR, S A¹; BECKER, T C A² and CASTRO-PRADO, M A A^{1, *}.

State University of Maringá ¹Department of Cell Biology and Genetics, ²Department of Clinical Analyses; Avenida Colombo 5790 - Maringá PR Brazil, 87020-900.

ABSTRACT

Mutations in the gene *uvsH* of *Aspergillus nidulans* result in increased spontaneous chromosome instability and increased intragenic and intergenic mitotic recombination in homozygous diploids. The aim of the present work was to obtain a *uvs* mutant of *A. nidulans* and to use it for the isolation of asexual recombinants (parameiotic segregants). The mutant *uvsH*, named B511, showed normal frequency of meiotic recombination in sexual crosses and high frequency of parameiotic segregants in the parasexual crossings with master strains (B511//A757 and B511//A288). Asexual haploid recombinants (parameiotic segregants), diploid and aneuploid segregants were recovered directly from the *uvs//uvs+* heterokaryons (B511//A757 and B511//A288). Parameiotic segregants originated through mitotic crossing-over and independent assortment of chromosomes.

Key terms: mitotic crossing-over, parameiosis, *uvs* mutation.

INTRODUCTION

Aspergillus nidulans is a homothallic fungus (i.e. it is self-fertile) with eight genetically defined chromosomes, which provides an excellent model for studying different aspects of developmental characters (Timberlake and Marshall 1988). The utilization of *A. nidulans* in genetic analysis has been improved by different life cycles in the ascomycete. Different from most filamentous fungi, *A. nidulans* shows three different proliferate cycles: asexual, sexual, and parasexual. Only the last two favour the occurrence of genetic variability (Baptista et al. 2003, Van de Vate and Jansen 1978, Kafer 1977, 1969, 1960, Pontecorvo and Roper 1952).

The parasexual cycle essentially involves the hyphal anastomosis of genotypically distinctive homokaryons, resulting in a heterokaryotic mycelium. The fusion of two unlike haploid nuclei results in a diploid nucleus. After undergoing mitotic crossing-over and/or mitotic non-

disjunction, the diploid nucleus may return to its haploid status through subsequent mitotic divisions (Debets 1998, Pontecorvo 1956). Parameiosis is a variation of the parasexual cycle in which genetic recombination and haploidization may occur inside the heterokaryotic hyphae, before conidia production. In fact, haploid recombinant segregants are originated directly from heterokaryons and the diploid phase is not recovered. The parasexual cycle with parameiosis constitutes an important pathway for studying the occurrence of genetic exchanges. In contrast to the long-term process required by a conventional parasexual cycle to form recombinant cells, parameiosis is a ready manner to evaluate mitotic crossing-over (Becker and Castro-Prado 2004, 2006, Baptista et al. 2003, Bello and Pacolla-Meirelles 1998, Bagagli et al. 1991, Bonatelli et al. 1983).

The analysis of mitotic recombination in *A. nidulans* has also been improved by *uvs* (sensitive to UV light) mutations, which

* Author for correspondence. State University of Maringá, Department of Cell Biology and Genetics. Avenida Colombo 5790, Maringá PR Brazil. 87020-900. e-mail: maacprado@uem.br - phone: (55) 44 3261-4679

change the normal frequencies of mitotic recombination in diploid cells (Baptista *et al.* 2003, Chiuchetta and Castro-Prado 2002, Osman *et al.* 1991). Mutations of two *uvs* epistatic groups, *UvsC* (*uvsA*, *uvsC* and *uvsE*) and *UvsF* (*uvsF*, *uvsH* and *uvsJ*) have been reported to be associated with the post-replication repair process (Goldman *et al.* 2002). The *uvsH* mutation actually shows homology with *Saccharomyces cerevisiae* *RAD18* and *Neurospora crassa* *uvs-2* mutations. Haploid *nuvA* (*uvsH*) mutants are not merely slow growing and have a “crinkly” morphology, but are also highly sensitive to UV light and to methyl-methane-sulphonate (MMS). *nuv//nuv* diploid mutants have significantly increased frequencies of mitotic recombination (Iwanejko *et al.* 1996, Osman *et al.* 1991).

Since *A. nidulans* spends most of its cell cycle in G2 phase (Osman *et al.* 1993), which significantly favors the event of mitotic recombination once the

chromosomes become duplicated in this phase (Kafer and May 1998, Osman *et al.* 1993), current research aims to obtain an *A. nidulans uvsH* mutant and to apply it to obtain parameriotic segregants (asexual recombinants).

MATERIAL AND METHODS

Media and Aspergillus nidulans strains: Complete (CM) and Minimal Medium (MM) have been previously described (Van de Vate and Jansen 1978, Pontecorvo *et al.* 1953). Selective medium (SM) consisted of MM supplemented with the nutritional requirements of the crossing strains with the omission of one of them, in each type of medium. Solid medium contained 1.5% agar. Triton X100 (0.01%) (TSM) was used to obtain compact colonies, while *Aspergillus nidulans* strains are described in Table 1.

TABLE 1

Genotypes of *A. nidulans* strains

Strains	Genotype	Origin
A837	<i>pabaA1</i> (I), <i>uvsH77</i> (IV), <i>pyroA4</i> (IV), <i>choA1</i> (VII), <i>chaA1</i> (VIII)	FGSC
A495	<i>lysB5</i> (V), <i>nicA2</i> (V), <i>pA2</i> (V)	FGSC
A288	<i>yA2</i> (I), <i>adE20</i> (I), <i>su adE20</i> (I); <i>wA3</i> (II); <i>galA1</i> (III); <i>pyroA4</i> (IV); <i>facA303</i> (V); <i>sB3</i> (VI); <i>nicB8</i> (VII); <i>riboB2</i> (VIII)	FGSC
A757	<i>yA2</i> (I), <i>methA17</i> (II), <i>pyroA4</i> (IV)	FGSC
A610	<i>yA2</i> (I), <i>pabaA1</i> (I).	FGSC
A507	<i>FpaB37</i> (I), <i>SulA1</i> (I), <i>anA1</i> (I), <i>pabaA1</i> (I)	FGSC
UT448	<i>wA2</i> (II), <i>riboA1</i> (VIII), <i>pabaA124</i> (I), <i>biA1</i> (I), <i>AcrA1</i> (II)	Utrecht
UT184	<i>SulA1</i> (I); <i>AcrA1</i> (II); <i>galA1</i> (III); <i>pyroA4</i> (IV); <i>facA303</i> (V); <i>lacA1</i> , <i>sB3</i> (VI); <i>nicB8</i> (VII); <i>riboB2</i> (VIII), <i>chaA1</i> (VIII)	Utrecht
B211	<i>yA2</i> (I); <i>biA1</i> (I); <i>wA2</i> (II); <i>AcrA1</i> (II); <i>methA17</i> (II); <i>pyroA4</i> (IV); <i>uvsH77</i> (IV); <i>chaA1</i> (VIII)	MGL
B520	<i>pabaA124</i> (I); <i>biA1</i> (I); <i>methA17</i> (II)	MGL
B511	<i>pabaA1</i> (I); <i>uvsH77</i> (IV), <i>lysB5</i> (V), <i>nicA2</i> (V), <i>pA2</i> (V), <i>chaA1</i> (VIII)	MGL

Mutant alleles have the following phenotypes: *pyro*, *lys*, *nic*, *sB*, *ribo*, *paba*, *bi*, *meth*, *cho*, *an*, *ad*, *pro*: requirements for pyridoxine, lysine, nicotinamide, thiosulfate, riboflavine, paraminobenzoic acid, biotin, methionine, choline, aneurine, adenine and proline, respectively; *y*, *w*, *cha*, *p*: yellow, white, chartreuse and pale, conidial color, respectively; *Acr*, *Fpa*, *Sul* determine resistance to Acriflavine, *p*-fluorophenylalanine and sulphanilamide, respectively; *gal* and *fac*: unable to grow on galactose and acetate as a carbon source respectively; *su* suppressor of *ad* allele; *uvs*: sensibility to UV light. FGSC, Fungal Genetic Stock Center, Kansas, USA; MGL, Microorganisms Genetic Laboratory, State University of Maringá.

Genetic techniques: The general methodology followed those previously described (Pontecorvo et al. 1953, Roper 1952). Heterokaryons were prepared in liquid MM plus 2.0% CM. After 21 days of incubation at 37°C cleistothecia were obtained from heterokaryons and the ascospores were inoculated in CM plates for further analysis.

Phenotype analysis of segregants: Aliquots of ascospores suspension of a hybrid cleistothecium from A837 x A495 cross were inoculated in CM plates to determine the phenotype of meiotic segregants. Progeny was isolated and inoculated in CM plates containing 25 pre-defined positions (master plates). The phenotype of each segregant was determined by inoculating them in different SM. Only those that demonstrated *paba*, *lys*, *nic*, phenotype were selected for UV-sensitivity test.

Conidia suspension: Conidia of each strain were inoculated in CM plates and incubated for 5 days at 37° C. Plate surface was washed with Tween 80 (0.001%); conidia suspension was filtered, washed by centrifugation and stored at 5°C in NaCl (0.85%) before treatments. The number of conidia was determined by haemocytometer counts.

UV-sensitivity test: Conidia of each meiotic segregant were inoculated in TSM for UV-sensitivity test. Plates were irradiated for periods of 0, 5, 10, 20 and 40 seconds in a UV-camera, using a Philips 30W germicide light, at a distance of 55 cm (UV dose rate reached 1.4 ergs.sec⁻¹). Experiments were carried out in the dark to prevent photoreactivation.

Obtaining asexual parameiotic recombinants from heterokaryons: Conidia of crossed strains were inoculated together in liquid medium to form heterokaryons. Heterokaryons constituted by *uvs*^{+/+}/*uvs*⁺ and *uvsH*/*uvs*⁺ crosses were inoculated in different selective media as shown in Table 2. Parameiotic segregants were identified as homogeneous and vigorous sectors growing from heterokaryons as shown in Fig. 2.

Analysis of Parameiotic Segregants: To determinate the mitotic stability of parameiotic segregants they were

inoculated in MC + benomyl (0.5 mg/L) plates. Stable segregants were crossed with master strains for the analysis of meiotic segregation of genetic markers. Results were compared using a 2x2 contingency table Yates' Corrected X²- test (p<0, 05).

TABLE 2

Selective media for the inoculation of heterokaryons

Crosses	Selective Media
B511//A288	MM, MM + <i>pyro</i> + <i>ribo</i>
B511//A757	MM + <i>meth</i> + <i>lys</i> , MM + <i>meth</i> + <i>lys</i> + <i>nic</i> , MM + <i>paba</i> + <i>pyro</i>
A288//A757	MM + <i>ad</i> + <i>pyro</i> + <i>ribo</i> MM + <i>pyro</i> + <i>nic</i> + <i>ribo</i> MM + <i>pyro</i> + <i>sB</i> + <i>ribo</i> MM + <i>ad</i> + <i>pyro</i> + <i>nic</i> MM + <i>pyro</i> + <i>ribo</i> MM + <i>pyro</i>

MM, Minimal medium; *meth*, *lys*, *paba*, *nic*, *ribo*, *pyro*, *ad* and *sB* are methionine, lysine, paraminobenzoic acid, nicotinamide, riboflavine, pyridoxine, adenine and thiosulfate nutritional requirements, respectively.

RESULTS AND DISCUSSION

Analysis of meiotic progeny from A837 x A495 cross showed that four segregants were obtained with nutritional requirements for paraminobenzoic acid, lysine and nicotinamide (results not shown). Only B511 strain, with genotype: *chaA1* (VIII), *pA2* (V), *pabaA1* (I), *uvsH77* (IV), *lysB5* (V) and *nicA2* (V), was sensitive to UV light as shown in Figure 1.

Previous researches demonstrate that meiotic recombination frequencies, in *uvsH* homozygous crosses, corresponded to those observed in both *uvsH* x *uvs*⁺ and *uvs*⁺ x *uvs*⁺ crosses (Iwanejko et al. 1996, Kafer and Mayor 1986). In current research and according to the literature, meiotic homozygous cross for *uvsH* mutation (B511 x B211) matched the recombination frequencies observed for heterozygous (*uvs*⁺ x *uvsH*) and homozygous (*uvs*⁺ x *uvs*⁺) crosses (Table 3).

Figure 1: Survival curve of B520 (*uvr+*), A837 (*uvrH*) and B511 (*uvrH*) strains during 5, 10, 20 and 40 seconds exposition to UV light.

Figure 2: Mitotic segregants obtained from heterokaryon B511//A757: **A:** 1- paramirotic (haploid) and 2 - heterokaryotic sectors; **B:** paramirotic P7 segregant (arrow); Mitotic segregants obtained from heterokaryon B511//A288: **C:** 1 – paramirotic (P10) and 2 – aneuploid segregants; **D:** arrows show aneuploid segregants.

TABLE 3

Meiotic segregation of genetic markers of chromosomes I, II, IV and VIII and frequencies of recombination between markers in chromosomes I and II of B511 x A288 and B511 x B211 crosses.

Meiotic Crosses	Segregation of Genetic markers								
	<i>ribo⁺:ribo</i>	<i>paba⁺:paba</i>	<i>y⁺:y</i>	<i>bi⁺:bi</i>	<i>Acr⁺:Acr</i>	<i>w⁺:w</i>	<i>meth⁺:meth</i>	<i>pyro⁺:pyro</i>	<i>cha⁺:cha</i>
Control	108:109 ^a	117:100 ^a	61:54 ^a	107:110 ^a	100:117 ^a	102:115 ^a	105:112 ^a	57:41 ^b	49:49 ^b
B511 x A288	46:38	37:45	10:6	-	-	39:43	-	42:40	22:17
B511 x B211	-	56:44	26:27	42:58	51:49	53:47	52:48	48:52	-

Meiotic Crosses	Frequencies of recombination (%)			
	<i>paba-bi</i>	<i>paba-y</i>	<i>y-bi</i>	<i>Acr-w</i>
Control	23.4 (34/145) ^a	14.1 (12/85) ^a	7.0 (6/85) ^a	28.2 (41/145) ^a
B511 x A288	-	17.9 (7/39)	-	-
B511 x B211	14.0 (14/100)	11.3 (6/53)	13.2 (7/53)	24.0 (24/100)

^a A757 x A757; ^b A507 x UT184; (-) Not analysed.

Asexual parametiotic recombinants, diploid and aneuploid segregants were recovered from heterokaryons of heterozygous *uvsH* crosses (B511//A288 and B511//A757) (Table 4, Fig. 2). The obtaining of parametiotic segregants may be explained by the occurrence of independent assortment of chromosomes or mitotic crossing-over and haploidization of diploid nuclei inside the heterokaryotic hyphae. On the other hand, aneuploid segregants may have been the result of unstable diploid nuclei that did not attain their haploid condition (Bello and Pacolla-Meirelles 1998, Bagagli et al. 1991, Osman 1991).

Our results demonstrate in Table 4 that

heterokaryons formed with the B511 mutant strain were able to produce, by parasexual cycle, a higher number of parametiotic segregants (asexual recombinants) than the A288//A757 control cross. Recombinant parametiotic segregants were produced by mitotic crossing-over (P2, P5, P7, P8, P10 and P11) and by independent chromosomal assortment (P1, P3, P4, P6 and P9) (Table 5). Since *uvsH* mutation actually increases the frequencies of intra- and intergenic mitotic recombination and alters chromosomal segregation in mitosis (Kafer and May 1998, Osman 1991), the isolation of mitotic recombinants has been facilitated by B511 strain.

TABLE 4

Mitotic segregants from *uvs⁺//uvs⁺* and *uvsH//uvs⁺* heterokaryons

CROSSES	H	P	D	A	PS	PS/H (%)	T
A288//A757	24	3	0	7	0	0	10
B511//A757	16	3	0	8	9*	56.3	20
B511//A288	8	0	2	15*	2	25.0	19

H, heterokaryons; P, parental; D, diploids; A, aneuploids, PS, parametiotic segregants; T, total of segregants. (*) Significantly different from A288//A757 control cross, 2x2 contingency Table, Yates' Corrected Chi-square test, $p < 0.05$.

TABLE 5

Phenotype of parameiotic segregants recovered from B511//A288 and B511//A757 crosses

Parameiotic Segregants	Phenotype	Origin	Crosses
P1	<i>y, uvs</i>	ia	B511//A757
P2	<i>cha, meth</i>	mco	B511//A757
P3	<i>y, uvs</i>	ia	B511//A757
P4	<i>y, uvs, cha</i>	ia	B511//A757
P5	wild-type	mco	B511//A757
P6	<i>y, uvs, cha</i>	ia	B511//A757
P7	wild-type	mco	B511//A757
P8	wild-type	mco	B511//A757
P9	<i>y, uvs, cha</i>	ia	B511//A757
P10	<i>w⁺, y</i>	mco	B511//A288
P11	<i>cha</i>	mco	B511//A288

(ia) independent assortment of chromosomes; (mco) mitotic crossing-over.

Mitotic stability of parameiotic segregants was observed in presence of benomyl. Normal frequencies of recombination and Mendelian segregation of genetic markers were observed in meiotic crosses of parameiotic segregants with master strains, as shown in Table 6. P1, P7, P8 and P9 were self-crossed and the ascospores only gave origin to prototrophic segregants (results not shown). Results reveal the genetic and chromosomal stability of parameiotic segregants or asexual recombinants.

Mitotic recombination may results from spontaneous chromosomal exchanges or may be induced by both physical and chemical agents. The process favors the expression of deleterious genes previously present in heterozygous condition through the origin of clones of cells homozygous for the distal genes to the point of exchange (Hagstrom and Dryja 1999, Beumer et al. 1998, Pires and Zucchi 1994).

TABLE 6

Meiotic segregation of genetic markers from parameiotic segregants crossed with master strains, and frequencies of meiotic recombination of P3 x UT448 and P4 x B520 crosses.

Meiotic Crosses	Segregation of Genetic markers							
	<i>ribo⁺:ribo</i>	<i>paba⁺:paba</i>	<i>y⁺:y</i>	<i>Acr⁺:Acr</i>	<i>w⁺:w</i>	<i>meth⁺:meth</i>	<i>pyro⁺:pyro</i>	<i>cha⁺:cha</i>
Control	108:109 ^a	117:100 ^a	61:54 ^a	100:117 ^a	102:115 ^a	105:112 ^a	57:41 ^b	49:49 ^b
P2 x B520	-	59:65	-	-	-	-	-	69:55
P3 x UT448	73:77	77:73	41: 36	63: 87	77: 73	-	-	-
P4 x B520	-	63: 62	58: 67	-	-	64: 61	-	-
P5 x UT448	69: 56	61: 64	-	-	57: 68	-	-	-
P6 x B520	-	60: 64	52: 72	-	-	54: 70	-	74: 50
P10 x A610	-	50: 50	-	-	-	-	-	-
P11 x A610	-	51: 56	51: 56	-	-	-	-	-

Meiotic Crosses	Frequencies of recombination (%)	
	<i>paba-y</i>	<i>Acr-w</i>
Control	14.1 (12/85) ^a	28.2 (41/145) ^a
P3 x UT448	7.8 (6/77)	25.3 (38/150)
P4 x B520	11.2 (14/125)	-

(^a) A757 x UT448, (^b) A507 x UT 184, (-) not analysed.

Present research has shown the isolation of a *uvs* mutant of *A. nidulans* applicable to recombination studies. Strain B511 proved to be a useful tool for the production of stable parameiotic segregants.

ACKNOWLEDGEMENTS

We would like to thank CAPES (Nucleus for Upgrading University Personnel), CNPq (National Council for Scientific and Technological Development) and Fundação Araucária (Foundation for the Support of Research of the State of Paraná) for their support. Thanks are also due to Ms. Sônia Aparecida de Carvalho and Mrs. Luzia de Souza Regazzi for their technical assistance. To god, once more, our efforts.

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