

ISOLATION OF PUTATIVE RECOMBINATION MUTANTS OF *Aspergillus nidulans*

Tânia M.A. Domingues Zucchi

ABSTRACT

A method was devised for the detection of mutants which produce altered frequencies of recombinants. Inspection of ascospore colonies, on a selective medium, can detect mutations (in one parent) that appear to affect recombination in the *meth A - w A* interval of linkage group II of *Aspergillus nidulans*, an interval which yields 1% recombinants.

Isolates resulting from N-methyl-N'-nitrosoguanidine treatment were crossed individually to a *meth A17* strain. Using *meth* and *w* as markers, recombinants were detected by plating on selective medium, and classification for conidial color. The method has revealed several classes of mutants, including those showing meiotic recombination frequencies different from normal.

INTRODUCTION

Catcheside (1975) demonstrated that most of the variation in crossing over frequency observed in strains of *Neurospora crassa* and related species can be attributed to genetic functions controlling the exchanges at specific regions of the genome. It is also known that dominant alleles of these genes cause changes in recombination frequency.

The need to understand the genetic factors controlling meiotic and mitotic crossing-over, gene conversion and repair in several organisms has produced growing interest in the selection of mutants with altered recombination frequencies. Most of the systems used to detect the mutations affecting meiotic events are directed to the isolation of mutants showing high or low recombination frequencies in the entire genome or at specific sites.

Good methods are currently available for the study of the phenomenon of recombination in *Aspergillus nidulans*: a) study of natural alleles promoting recombination; b) deletion of elements controlling recombination in I-II duplication; c) alteration of recombination between close mutant alleles. (Parag, 1962; Shanfield and Käfer, 1969; Jansen, 1970; Fortuin, 1971a,b,c; Van de Vate and Jansen, 1978).

The last method (c) was used in the present study. The system chosen was that of *meth-w* mutant alleles, since it permits rapid detection of recombinants in the *meth* A17 - *w* A2 interval of chromosome II, within which the normal meiotic recombination frequency is around 1%.

MATERIAL AND METHODS

Strains

Following Clutterbuck's proposals (1970), the mutant alleles of the *A. nidulans* strains used in the present study were:

UT 448: (Utrecht stock): *w* A2 (II) white conidia; *ribo* A1, *paba* A124, *bio* A1 (I) with requirements for riboflavine, *p*-aminobenzoic acid, and biotin, respectively; *acr* A1 (II) resistance to acriflavine.

UT 196: Tester strain: *y* A2 (I) yellow conidia; *meth* A17 (II); *pyro* A4 (IV) with requirements for methionine and pyridoxine, respectively (Figure 1).

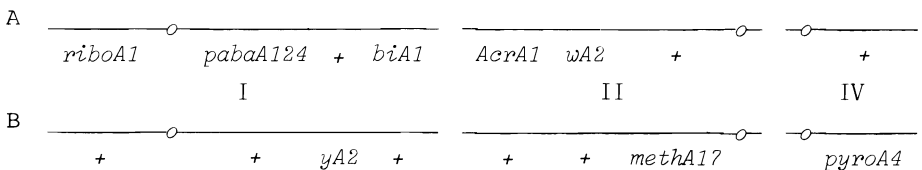


Figure 1 - Genotype of the strains used; 1A: UT 448 strain; 1B: UT 196, tester strain. The centromeres are represented by open circles. The positions of the markers are not presented in scale.

Media and solutions

Complete (CM) and *minimum media* (MM) were as described by Van de Vate and Jansen (1978).

MNNG solution: the solution was prepared in Tris G-3.300-malate buffer, pH 9.0, at a concentration of 0.5 mg MNNG/ml. The solution was homogenized by sonication for five minutes (MNNG: N-methyl-N'-nitro-N-nitrosoguanidine).

Selective medium for crosses (SM): 200 ml of MM was supplemented with 0.2 mg of riboflavine; 0.004 mg of biotine; 200 mg of casaminoacid, and 1.0 mg of adenine.

Selective medium for mutant isolation (S1): 200 ml of MM (liquid) supplemented with 0.2 mg of riboflavine; 0.004 mg of biotine; 0.5 μ g of pyridoxine; and 1.0 mg of sodium deoxycholate to produce small (compact) colonies.

Preparation of conidial suspensions: conidia of UT 448 and UT 196 were collected from CM plates by washing the surface twice with 10.0 ml of saline (0.85% v/v) - tween 80 (0.1%) and rubbing with a glass rod to liberate the conidia. The suspension was filtered through a cotton filter, pre-washed with saline. All these steps were done under aseptic conditions. The filtrate was centrifuged at 3,000 rpm for 15 minutes and washed twice with 15.0 ml saline, transferred to vials and dispersed by sonication for 15 minutes.

Mutagenesis

The method used consisted of the following steps: a) a *meth*⁺ strain was treated with nitrosoguanidine (N-methyl-N'-nitro-nitrosoguanidine); b) survivors were individually crossed with a tester *meth* strain; c) a large cleistothecium from each cross was checked to determine if there were alterations in recombination frequency. It is well known that MNNG decomposes rapidly into an alkaline solution. In such a situation mutagenesis is quantitatively related to the decomposition of MNNG into an unstable and mutagenic compound, which is believed to be diazomethane (Delic, Hopwood and Friend, 1970). Since mutagenesis is correlated to MNNG decomposition and there is no evidence that the non-decomposed MNNG has mutagenic effects, the method used here is very adequate and safe.

Mutagenesis of the UT 448 strain: 5×10^5 conidia/ml of the UT 448 strain were added to small vials containing the MNNG buffered solution. A buffered control was prepared with the same conidial concentration. Samples of both suspensions (control and treatment) were collected to determine the number of viable conidia through dilution and plating on CM + SD. After 48 h at 37°C the colonies were scored.

Both suspensions (control and treatment) were incubated at 37°C with shaking for 6 hours. The number of viable conidia and the percent survivors were determined.

Crosses: to obtain 10 to 20 colonies per plate, the UT 448 conidial suspension was diluted and seeded on CM plates. The preparation was incubated for three days at 37°C. Conidia from the edge of each isolated colony were individually transferred to 9 defined positions (3 x 3 pattern) on CM plates.

After three days of incubation at 37°C, the colonies were replicated to SM plates with 1×10^7 conidia of UT 196, seeded in advance with SM top-layer agar. After 48 h at 37°C the plates were sealed with adhesive tape and incubated again for 16 days.

Spot test: Fast analysis of recombination frequencies in the interval *paba-y* (I) and *meth-w* (II). After 18 days of incubation the heterokarya formed at each inoculation point produced many cleistothecia. A large one was chosen and roughly cleaned in 3% agar. The cleistothecium was crushed against the wall of the tube containing melted MM top layer agar, in order to liberate the ascospores, and diluted successively at: 1:10, 1:20 and 1:50 in melted MM top layered into tubes. The contents of each tube was then poured onto a plate with S1 media. After spreading, the plates were left to stand for 15 min. until the top layer had settled, and then incubated at 37°C for three days. The colonies were scored on diluted plates and the spots (*i.e.* the yellow and green colonies) were scored from the undiluted plates. S1 + SD medium selects against *meth* and *paba* and the growing *meth*⁺ *paba*⁺ are all recombinants. The spots were mainly *y* since *y*⁺ is linked to *paba*. The absolute number of spots measures the *meth-w* recombination and the yellow-green ratio indicates *paba-y* recombination. Since the number of ascospores varies widely among the different crosses, the spots should be counted in relation to the total number of colonies (if the recombination was suspected to be altered). If the frequency found is different from the control (1%), further tests are needed (Figure 2).

The suspensions of treated conidia were kept at 7°C throughout the period of study (several months) while the other steps were performed. Since during this period conidial survival decreases about 80%, immediately after treatment the conidia should be washed to avoid the effects of MNNG decomposition.

The survivors were crossed in series of 20 to 120 crosses each. The series were performed (I, II, III, ... X) for a total of 862 crosses, 663 of which were fertile, 161 sterile and 38 were discarded because they were selfed or could not be counted.

RESULTS

The spot test results are presented in Figures 3 and 4.

The most promising mutants selected from the crosses were in the classes whose recombination frequencies differed from normal, *i.e.* more than 4% and less than 1% (Table I). Among these, mutant candidates with a high *meth*⁺ *w*⁺ recombinant frequency, normal morphology and normal conidiation were again tested and three or more cleistothecia of each were analyzed (Table II). Only the mutants that maintained abnormal recombination frequencies were selected for further analysis.

Few of the many initially promising candidates were utilized. The mutants with low recombination frequencies are not discussed here since their very narrow *meth-w* distances (1-2%), made analysis very difficult. Furthermore the results between 0 and 1% cannot always be regarded as abnormal in relation to recombination in that interval.

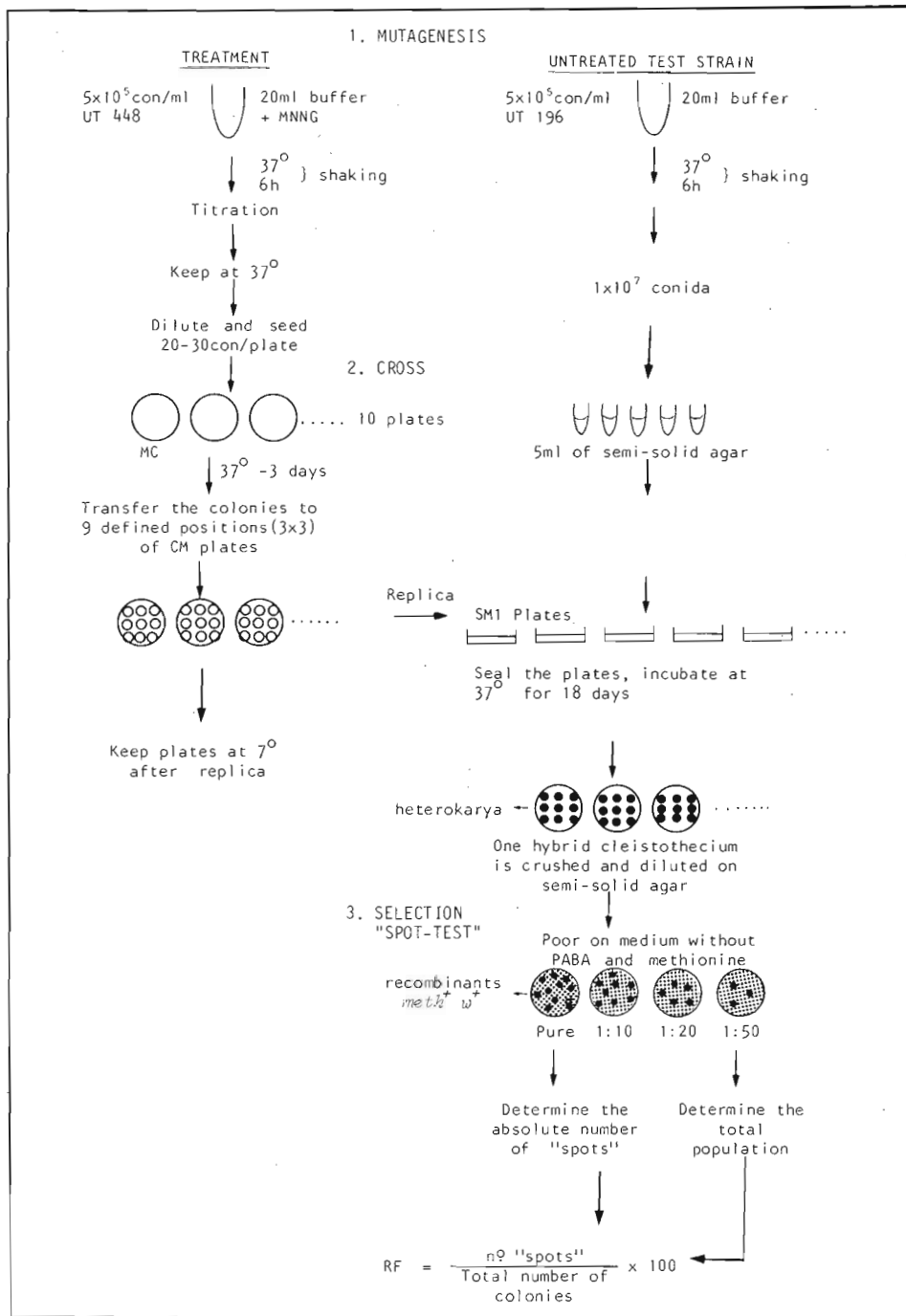


Figure 2 - Flow diagram of spot test.

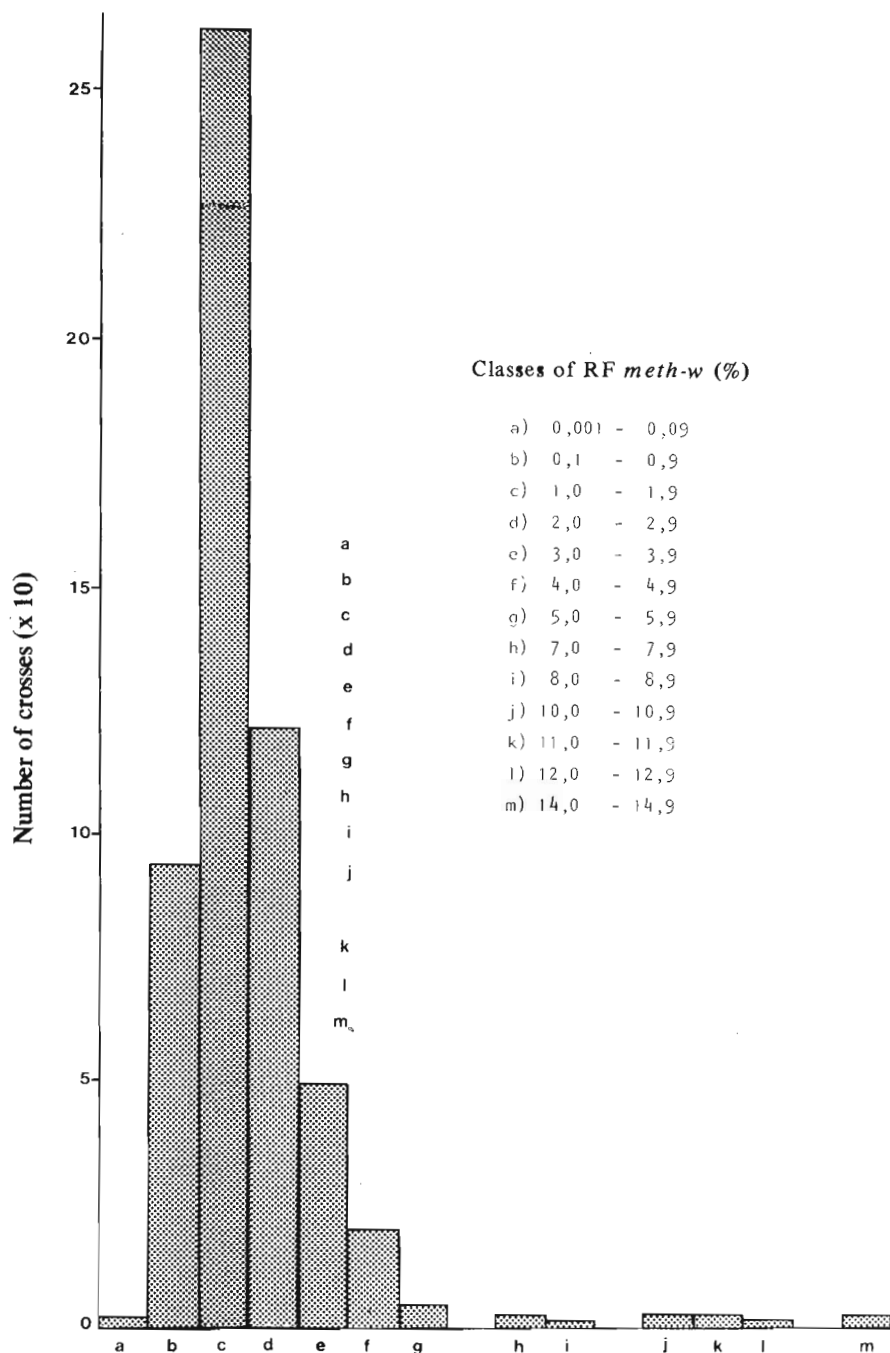


Figure 3 - *meth-w* recombination frequencies (RF) presented by the UT 448 survivors after MNNG treatment and crossed with the UT 196 strain.

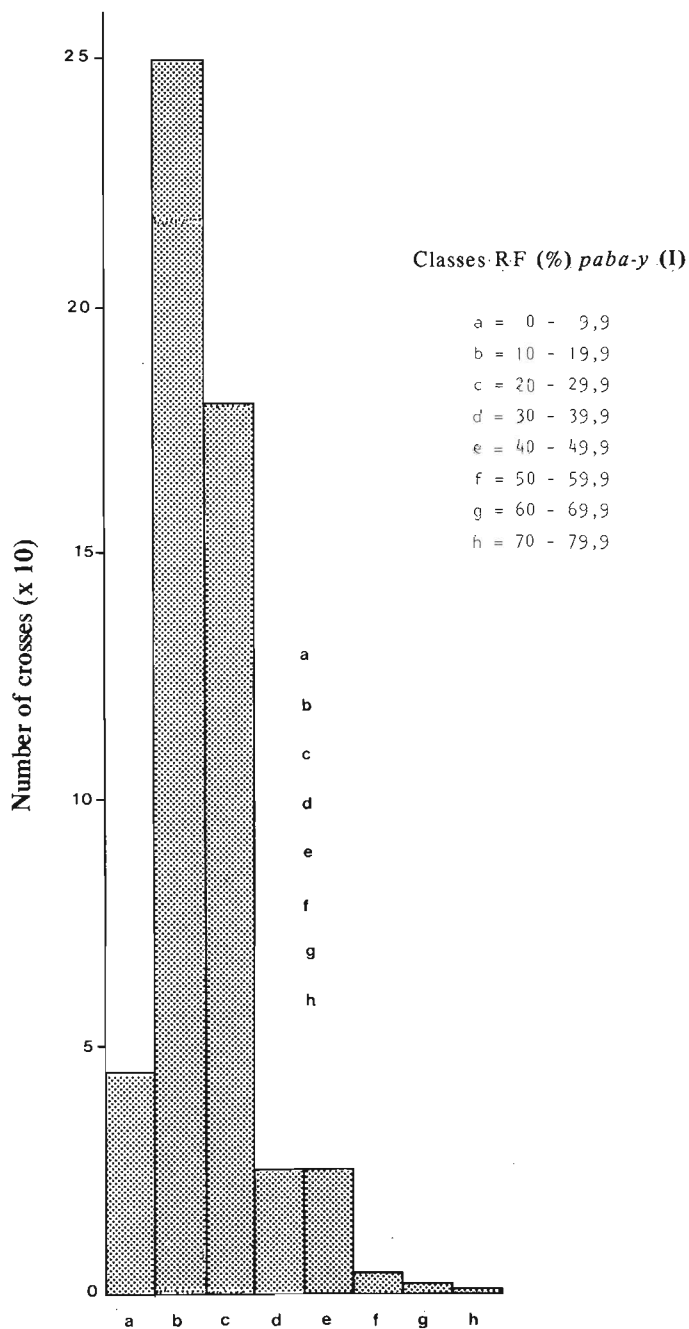


Figure 4 - *paba-y* recombination frequencies (RF) presented by the UT 448 survivors after MNNG treatment and crossed with UT 196.

Table I - Frequency of w^+ $meth^+$ independent progeny from crosses between the best candidates and the tester strain (UT 196) (Just one cleistothecium from each cross).

High frequency (> 4%)			Low frequency (< 1%)	
Code		R.f. <i>meth-w</i> (%)	Code	R.f. <i>meth-w</i> (%)
29 VI	(11,000)	14.9	52 VIII	(50) 0.04
66 V	(272)	14.7	45 XI	(3,200) 0.06
11 IX	(17,750)	12.3	99 IX	(7,150) 0.07
30 VIII	(17,750)	8.1	91 VIII	(9,800) 0.19
32 VII	(13,200)	8.0	118 V	(5,040) 0.23
116 V	(13,360)	7.0	33 VI	- 0.26
24 VIII	(16,500)	5.7	4 I	(3,920) 0.36
38 VIII	(9,400)	5.6	23 VII	(15,900) 0.36
56 VIII	(28,950)	5.5	11 VII	(30,600) 0.44
58 VIII	(19,450)	5.1		
61 VIII	(34,500)	4.5		
29 VII	(16,200)	4.2		
85 II	(9,150)	4.4		

The total of population is given in parentheses.

Table II shows that only a few mutant candidates kept the initial alterations in the RF *meth-w*. The new selection considerably reduced the initial number of candidates to 29 VI, 30 VIII, 24 VIII, 38 VIII and 118 V.

We also checked mitotic instability (Table III). Several lines of evidence have been previously presented (Baker *et al.*, 1976), concerning the frequency of this phenomenon and its presence in several recombination mutants. In general, mitotic instability is related to an excess of genetic material (Bainbridge and Roper, 1966; Nga and Roper, 1968; Van de Vate *et al.*, 1978; Zucchi and Azevedo, 1979; Marin, 1983). If $meth^+$ duplication is responsible for mitotic instability and alterations in the frequency of recombination, the spontaneous loss of the duplication should produce improved sectors and, consequently, *meth-w* recombination should return to a normal level.

Table II - "Spot test" results of some mutant candidate crosses. Control cross: 448 x 196.

Crosses	Number of crosses	RF <i>meth w</i> (M) %
448 x 196 (Control cross)	9	2.49 -
29 VI x 196	7	15.4 **
30 VIII x 196	15	14.1 **
38 VIII x 196	11	7.3 **
30 VII x 196	4	5.3 **
58 VIII x 196	6	2.3 ns
23 VII x 196	5	0.69 ns
118 V x 196	5	1.6 ns
11 IX x 196	6	3.9 ns
66 V x 196	3	6.2 **
56 VIII x 196	7	2.5 ns
116 V x 196	3	3.4 ns
52 VIII x 196	2	1.3 -
4 I x 196	6	1.6 ns
24 VIII x 196	2	10.0 -

* and **: significant at 5% and 1% probability, Tukey test, comparing RFs with the control cross; ns: not significant.

Table III - Number of sectors per colony.

Strains	Improved (I)	Deteriorated (D)	Sectors/colony
448 (10)	0	0	0
30 VIII (10)	2	1	0.3
29 VI (10)	0	2	0.2
38 VIII (10)	4	5	0.9
24 VIII (10)	0	0	0
118 V (90)*	5	5	0.1

Note: The number of colonies tested is given in parentheses; * Results from Marin (1983).

On this basis, some recombination analyses of improved sectors of the mutants were conducted and the results are shown in Table IV. As can be seen, improved sectors from the 30 VIII and 38 VIII strains crossed with UT 196, produced large number of *meth*⁺ *w*⁺ recombinants eliminating at least temporarily the pos-

sibility that a *meth*⁺ duplication is responsible for the high RF *meth-w*. Requirement analysis also revealed that 38 VIII and its spontaneous sectors are adenine deficient, more specifically *ade* mutants. To locate this new mutation, a complete meiotic analysis was performed through the 38 VIII x UT 196 cross, and part of the results are presented in Table V. As can be observed, the new *ade* deficiency is on chromosome II, close to *meth* (17%) and *w* (15%). The *ade* mutation, at this position, explains well the high RF *meth-w* found in the spot-test (Table I): the S1 medium, used in the spot-test, selects against *ade* recombinants since this medium does not have adenine. In this way, the number of white segregant colonies is lower than in crosses involving normal strains (*w* is closely linked to *ade*) and since the calculated RF in the spot test is given by

$$\text{RF} = \frac{\text{yellow} + \text{green}}{\text{yellow} + \text{green} + \text{white}} \times 100 ,$$

reducing the number of *ade w meth*⁺ (white colonies), the RF *meth-w* will apparently be high.

Table IV - Recombination frequency in the *meth-w* interval of chromosome II of the improved (I) sectors derived from 30 VIII and 38 VIII mutants (spot test).

	I ₁ 30 VIII	I ₂ 30 VIII	I ₁ 38 VIII	I ₂ 38 VIII	I ₃ 38 VIII	I ₄ 38 VIII
Crosses	x	x	x	x	x	x
	196	196	196	196	196	196
	(10,480)	(8,860)	(3,340)	(6,320)	(4,700)	(5,880)
RF (%)	30.3	21.9	10.8	10.1	8.9	6.8

The total number of analyzed colonies are presented in parentheses.

When the selective medium for meiotic analysis is supplemented with adenine, the RF *meth-w* of 38 VIII returns to normal. For this reason such apparent *hyper-rec* mutants were discarded from our analysis.

This and other examples justify the need for a very judicious analysis of the selected recombination mutants; since there are several factors affecting the recombination frequency, even though they are not directly related to it. There is no relationship between *ade* requirements and high mitotic instability since this analysis was performed on CM that contained sufficient nucleic acid and adenine.

Table V - Meiotic analysis of 38 VIII x UT 196 (100 colonies were analysed).

Interval of chromosome II RF (%)	Parental class		Recombinant class	
	<i>w meth</i> ⁺	<i>w</i> ⁺ <i>meth</i>	<i>w meth</i>	<i>w</i> ⁺ <i>meth</i> ⁺
<i>meth-w</i> 2	48	50	0	2
	<i>meth</i> ⁺ <i>ade</i>	<i>meth ade</i> ⁺	<i>meth ade</i>	<i>meth</i> ⁺ <i>ade</i> ⁺
<i>ade-meth</i> 17	43	40	10	7
	<i>ade w</i>	<i>ade</i> ⁺ <i>w</i> ⁺	<i>ade w</i> ⁺	<i>ade</i> ⁺ <i>w</i>
<i>ade-w</i> 15	43	42	10	5

The other isolated mutant, 118 V, did not show a very high RF *meth-w* in the spot-test, but was deleted due to its high level of mitotic instability. The colonies sectored spontaneously and the improved sectors had normal RF *meth-w*, whereas the deteriorated sectors showed frequencies of around 25%. This peculiar meiotic behavior is due to the presence of a translocated segment bearing *meth*⁺ (Marin, 1983; Marin and Zucchi, in preparation) and *w* (Castro-Prado, 1986; Castro-Prado and Zucchi, in preparation). The 118 V mutant was designed by Z and will be discussed in a separate paper due to its complex characteristic.

Mutants 30 VIII, 29 VI, and 24 VIII showed that the apparent (not real) high RF *meth*⁺ -*w*⁺ was due to the action of silent *meth* A17 suppressor located on different chromosomes, i.e. VII, V and VI respectively. In some crosses anti-suppressor action on a non-related strain was detected (Zucchi, 1986).

The abnormal meiotic behavior of the chromosome II markers of these mutants is shown in Table VI. As can be observed, the control cross shows only two major classes, (1) and (2), whereas mutants 30 VIII, 29 VI and 24 VIII show 4 major classes: (1), (2), (3) and (4). 30 VIII, 29 VI and 24 VIII are also referred to as mutants 30, 29 and 24, respectively.

Table VI - Possible classes produced by exchanges between markers of chromosome II.

Classes	448 x 196	30 VIII x 196	29 VI x 196	24 VIII x 196
<i>Acr</i> + <i>w</i> (1)	111	94	223	87
+ <i>meth</i> + (2)	92	94	104	133
<i>Acr meth</i> +	18	24	3	11
+ + <i>w</i> (3)	22	84	99	22
+ <i>meth w</i>	0	0	0	0
<i>Acr</i> + +	2	7	38	6
+ + + (4)	3	83	102	39
<i>Acr meth w</i>	3	3	1	1

Partial genotype of crossed strains

II		

448, 30 VIII, 29 VI, 24 VIII		
<i>acr</i> A1	<i>w</i> A2	+

196		
+	+	<i>meth</i> A17

Growth curves of these mutants and are shown in Figure 5. It can be seen that the mutants grew slowly on CM and that growth of the 30 VIII mutant is the most affected by the mutation. Although the biological meaning of such unusual growth behavior is difficult to explain at this time, the fact is suggestive enough to merit further experiments.

Based on the results given in Table VII we can conclude that the high frequency of *meth*⁺ *w*⁺ recombinants presented in the spot test of 30 VIII, 29 VI and 24 VIII mutants must include the real *meth*⁺ *w*⁺ recombinants and a large number of *meth sup meth w*⁺ pseudo-recombinants.

DISCUSSION

In general, most of the recombination mutant selection systems involve the detection of prototrophic recombinants on selective medium, when the parental strains are heteroallelic for one marker and heterozygous for others. After the level of the prototrophic intragenic recombinants is determined, analysis of intergenic exchanges is performed. These systems, in general exclude mutations in genes that control intergenic exchanges (Baker *et al.*, 1976).

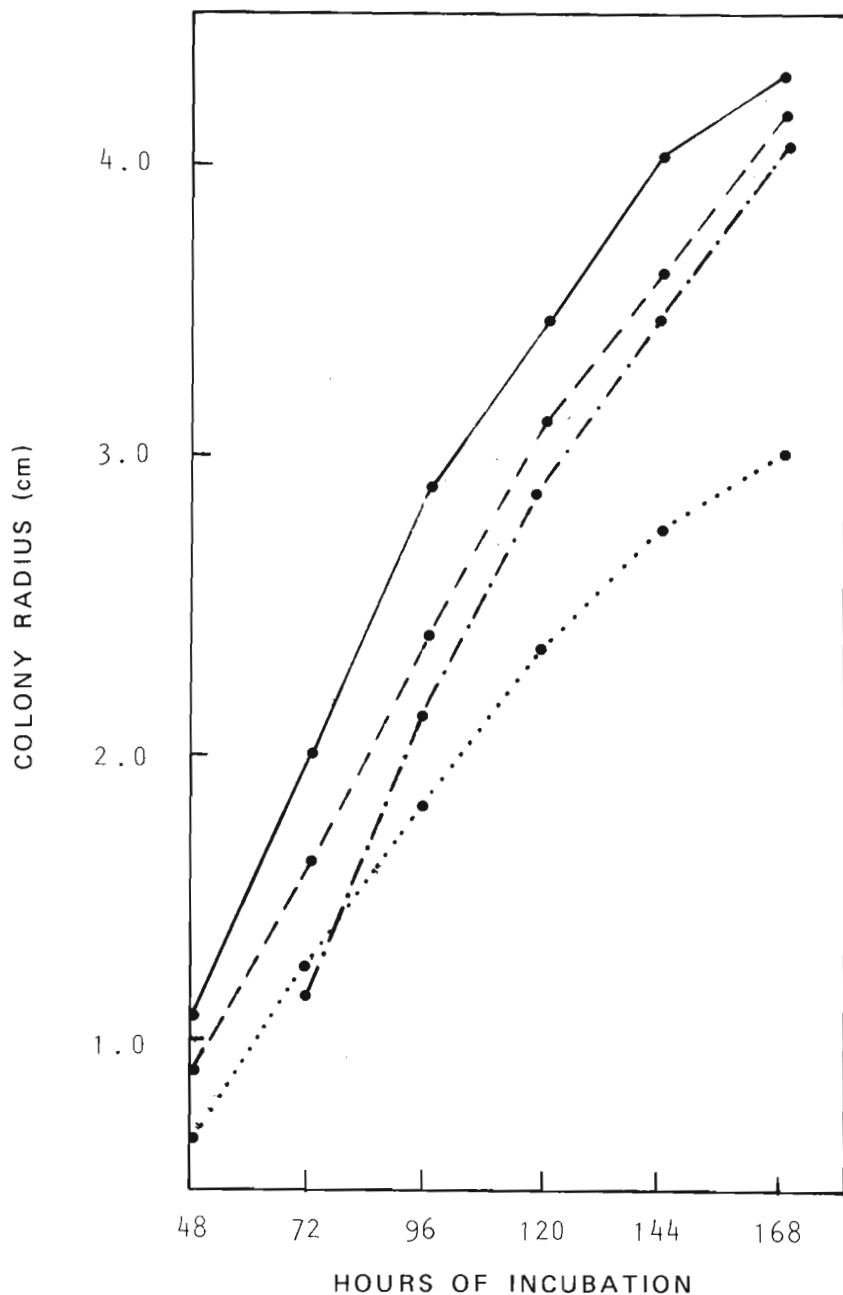


Figure 5 - Growth curves of the control strain UT 448 (—) and the mutants 29 VI (---), 24 VIII (-.-.), and 30 VIII (...). The conidia were inoculated into the center of CM plates and incubated at 37°C. Measurements (cm) were made every 24 h.

Table VII - *meth* segregation among the progeny of *meth*⁺ *w*⁺ recombinants crossed with a *meth*⁺ *w*⁺ normal strain.

<i>meth</i> ⁺ <i>w</i> ⁺ recombinants code	Origin	Crossed with UT 184 <i>meth</i> ⁺ <i>w</i> ⁺	
		<i>meth</i>	<i>meth</i> ⁺
1 ³		23	31
3 ⁶	30 VIII x 196	35	74
1A		17	83
1 ⁷	29 VI x 196	25	52
3 ¹		17	59
1 ⁹	24 VIII x 196	17	28
6 ¹⁹		27	108

Our method permits a fast and direct detection of strains showing abnormally high or low recombination frequencies on the *meth-w* interval of the II chromosome. A simple visual inspection of plates containing the colonies originated from ascospores of a cross is sufficient to detect the presence of mutations affecting the meiotic recombination frequency.

Induced mutations in the UT 448 strain are easily verified through an increase or reduction of the relative number of spots in medium lacking methionine and PABA. Although it concerns the recombination frequency, the method is not directed to the isolation of just one kind of alteration in DNA, structural or not, affecting crossing-over on *meth-w* or *paba-y* intervals.

Since the selective medium used (S1) lacks methionine and PABA, the mutations increasing *met*⁺ *w*⁺ and *pa*⁺ *y*⁺ recombinants are probably:

- a) suppressors of *meth* A17;
- b) suppressors of *paba* A124;
- c) lesions stimulating crossing-over at the *paba-y* interval or at the *ribo-paba* interval;
- d) lesions stimulating recombination at the *meth-w* interval;
- e) mutations affecting simultaneously the frequencies of recombinants *meth*⁺ *w*⁺ and *paba*⁺ *y*⁺;
- f) new auxotrophy, close to the tested segments;
- g) duplications of the *meth*⁺ segment.

Among these possibilities, the system allows one to select all the types of mutants.

Mutants *a*, *c* and *e* correspond to the *meth* suppressor mutants *sup* 30, *sup* 29 and *sup* 24 that confer the *hyper-rec* character to mutants 30 VIII, 29 VI and 24 VIII. The *d* type was recently found by Celere and Zucchi who used the spot test for selection of gamma radiation induced mutants.

The *f* type corresponds to the new auxotrophy linked to *meth* and presented by the 38 VIII mutant.

Finally, the mutation of the *g* type was that presented by 118 V, which has a *meth*⁺ and *w* duplication.

In spite of its time-consuming nature, the method used was considered very efficient for the selection of mutants with altered recombination frequencies in specific segments of the genome.

All the selected mutants were extensively studied after this preliminary analysis. Normally most of them showed more than one mutant *locus* affecting recombination frequency. The combination between them and interaction of their products showed interesting results to be presented in future papers.

ACKNOWLEDGMENTS

The financial support by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), turning our stay at the Utrecht State University (Holland) possible, is acknowledged. Special thanks are directed also to Dr. G.J.O. Jansen and C. van de Vate for research aid, useful discussions and partial revision of the text. We are indebted to the technical assistance of Mrs. Sonia Mathias da Silva.

Publication supported by FAPESP.

RESUMO

Foi estabelecido um método para detecção de mutantes que produzem frequências de recombinação alteradas.

A simples inspeção de colônias ascospóricas, em um meio seletivo pode detectar mutações (em um dos pais) que parecem afetar a recombinação no intervalo *met A-w A* do grupo de ligação II de *Aspergillus nidulans*, um intervalo que normalmente produz 1% de recombinantes.

Isolados do tratamento com N-metil-N'-nitrosoguanidina foram individualmente cruzados com uma linhagem *met A*₁₇. Usando *met* e *w* como marcadores, os recombinantes foram detectados pela sementeira em meio seletivo, e classificação para cor de conídio. O método mostrou várias classes de mutantes incluindo aqueles mostrando frequência de recombinação diferente das taxas normais.

REFERENCES

- Bainbridge, B.W. and Roper, J.A. (1966). Observations on the effects of a chromosome duplication in *Aspergillus nidulans*. *J. Gen. Microbiol.* 42: 417-424.
- Baker, B.S., Carpenter, A.T.C., Esposito, M.S., Esposito, R.E. and Sandler, L. (1976). The genetic control of meiosis. *Ann. Rev. Genet.* 10: 53-134.
- Castro-Prado, M.A.A. (1986). Estabilização de genes em dupla dose de mutantes hiper-rec-uvs *Aspergillus nidulans*. Masters Thesis, Faculdade de Medicina, USP, Ribeirão Preto, SP.
- Catcheside, D.G. (1975). Occurrence in wild strains of *Neurospora crassa* of genes controlling genetic recombination. *Aust. J. Biol. Sci.* 28: 213-225.
- Clutterbuck, A.J. (1970). *Aspergillus* symbols locus and letters and allele numbers. *Aspergillus Newsletter* 11: 25-33.
- Delic, V., Hopwood, D.A. and Friend, E.J. (1970). Mutagenesis by N-methyl-N'-Nitrosoguanidine (NTG) in *Streptomyces coelicolor*. *Mutat. Res.* 9: 167-182.
- Fortuin, J.J.H. (1971a). Another two genes controlling mitotic intragenic recombination and recovery from UV-damage in *Aspergillus nidulans*. I. *Mutat. Res.* 11: 149-162.
- Fortuin, J.J.H. (1971b). Another two genes controlling mitotic intragenic recombination and recovery from UV damage in *Aspergillus nidulans*. II. Recombination behaviour and X-ray sensitivity of uvs D and uvs E mutants. *Mutat. Res.* 11: 265-277.
- Fortuin, J.J.H. (1971c). Another two genes controlling mitotic intragenic recombination and recovery from UV damage in *Aspergillus nidulans*. IV. Genetic analysis of mitotic intragenic recombination from $uvs^+ uvs^+$, $uvsD/uvs D$ and $uvsE/uvs E$ diploids. *Mutat. Res.* 13: 137-148.
- Jansen, G.J.O. (1970). Abnormal frequencies of spontaneous mitotic recombination in uvs B and uvs C mutants of *Aspergillus nidulans*. *Mutat. Res.* 10: 33-41.
- Marin, J.M. (1983). Estudo de fatores genéticos que afetam a estabilidade mitótica e recombinação em *Aspergillus nidulans*. Masters Thesis, Faculdade de Medicina, USP, Ribeirão Preto, SP.
- Parag, Y. (1962). Mutations in the B incompatibility factor of *Schizophyllum commune*. *Proc. Nat. Acad. Sci. (Wash.)* 48: 743-750.
- Shanfield, B. and Käfer, E. (1969). UV-sensitive mutants increasing mitotic crossing-over in *Aspergillus nidulans*. *Mutat. Res.* 7: 487-489.
- Nga, B.H. and Roper, J.A. (1968). Quantitative intrachromosomal change arising at mitosis in *Aspergillus nidulans*. *Genetics* 58: 193-209.
- Van de Vate, C. and Jansen, G.J.O. (1978). Meiotic recombination in a duplication strain of *Aspergillus nidulans*. *Genet. Res. Camb.* 31: 29-52.
- Zucchi, T.M.A.D. and Azevedo, J.L. (1979). Mitotic instability in a III-VIII duplications strains of *Aspergillus nidulans*. *Rev. Bras. Genet.* 11: 93-108.
- Zucchi, T.M.A.D. (1986). Estudos de fatores genéticos que alteram as frequências de recombinação em *Aspergillus nidulans* (Eidam) Winter. "Livre Docência" Thesis. Faculdade de Medicina, USP, Ribeirão Preto, SP.