

## GENETIC ANALYSIS OF SOME FACTORS AFFECTING MITOTIC AND MEIOTIC BEHAVIOR OF A MUTANT OF *Aspergillus nidulans*

José Moacir Marin<sup>1</sup> and Tânia M.A. Domingues Zucchi<sup>2</sup>

### ABSTRACT

This study describes and characterizes a MNNG (N-methy-N'-nitro-N-nitrosoguanidine) induced mutant of *Aspergillus nidulans* presenting abnormal meiotic recombination frequencies in the *w-meth* interval of chromosome II. The mutant was selected on the basis of its unstable character, which originated spontaneous, improved or deteriorated sectors. Genetic analysis of several deteriorated sectors evidenced mitotic instability, morphological alterations UV sensitivity (*uvs*) and an apparent increase in the recombination frequency in the *w-meth* interval, suggesting a *meth*<sup>+</sup> duplication in the *paba-y* interval of chromosome I (*Dp* II-I). The *uvs* character and deteriorated morphology (*det*) of mitotic segregants seemed the direct outcome of the duplication insertion.

The significant increase in *meth-w* recombination frequency was related to the expression of the *meth*<sup>+</sup> character in the transposed element, since the spontaneous sectors, arising from deteriorated sectors, presented normal *w-meth* recombination frequencies, normal colony morphology and a completely restored mitotic stability.

### INTRODUCTION

The occurrence of improved or deteriorated sectors is common in strains of *A. nidulans* bearing duplicate chromosomal segments, one in the normal position and the other translocated to another linkage group. During vegetative growth, such strains may lose, totally or partially, the extra-segment. The improved sectors are the

---

<sup>1</sup> Departamento de Ciências Morfológicas, Faculdade de Odontologia-USP, 14049 Ribeirão Preto, SP, Brasil.

<sup>2</sup> Departamento de Parasitologia, Instituto de Ciências Biomédicas - USP, Av. Prof. Lineu Prestes, 1374, Cidade Universitária, 05508 São Paulo, SP, Brasil. Send correspondence to T.M.A.D.Z.

consequence of the loss, recovering the mitotic stability. The deteriorated and unstable sectors are the result of new *in tandem* duplication in one of the duplicate segments. Transposition of this new duplication to another region of the genome, confers some mitotic stability to the deteriorated strains (Nga and Roper, 1968; Azevedo and Roper, 1970).

Several authors suggest that insertion of an exogenous element in a different chromosomal region (e.g., a transposable element) promotes new genic rearrangements which affect the pattern of meiotic recombination (Nevers and Saedler, 1977). In some cases it is clear that duplication, mitotic instability and alterations in recombination frequency are the consequence of the same event.

The aim of this paper is two fold: first to show the effects of a *Dp* (II-I) in some deteriorated sectors derived from the Z mutant and, second, to determine the consequences of the spontaneous duplication loss.

The Z mutant is derived from the UT 448, mutagenized strain and presents an extra segment linked to its original position. This duplication bears the *meth*<sup>+</sup> marker and it is frequently transposed to different locations in the genome and may also be spontaneously lost.

## MATERIAL AND METHODS

*Strains*: the mutant alleles of the strains used were designated according to the nomenclature proposed by Clutterbuck (1981), as follows:

*UT 448*: *w* A2 (II) white conidia; *ribo* A1, *paba* A124, *bi* A1 (I), with requirements for riboflavin, *p*-aminobenzoic acid and biotin, respectively; *Acr* A1 (II) resistant to acriflavin.

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

*Z Mutant*: *w* A2 (II) white conidia; *ribo* A1, *paba* A124; *bi* A1 (I), with requirements for riboflavin, *p*-aminobenzoic acid and biotin, respectively; *Acr* A1 (II). This mutant presents *meth*<sup>+</sup> in duplication and linked to its original position (linkage group II).

*Media*: Minimal medium (MM) was Czapeck Dox with 1% (w/v) glucose. Complete medium (CM) contained yeast extract, hydrolyzed nucleic acids, vitamins, aminoacids, glucose (Pontecorvo *et al.*, 1953, modified by Van de Vate and Jansen, 1978). The solid medium contained 1.5% Difco Bact agar.

*Methods*: The general procedures followed those proposed by Pontecorvo *et al.* (1953).

Mitotic instability was demonstrated by sectors spontaneously appearing in colonies originating from single conidia after 7 days of incubation at 37°C.

The UV sensitivity test was performed by replicating master plates bearing the meiotic segregants obtained after crossing the sectors of the Z mutant with the UT 196 strain. Each master plate was replicated onto four complete medium plates which were treated as follows:

1. Not irradiated (control).
2. Without pre-incubation and irradiated for 30 sec.
3. Pre-incubated (8 h) and irradiated for 30 sec.
4. Pre-incubated (18 h) and irradiated for 30 sec.

All treated plates were incubated for 48 h at 37°C. UV irradiation was applied using a 15 Watt General Electric germicidal lamp G1578, located 40 cm from the plates in the absence of any other source of light.

The dosage of radiation was estimated to be 26 ergs/mm<sup>2</sup>/sec.

## RESULTS AND DISCUSSION

The UT448 strain was MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) treated and among others the Z mutant was selected (Zucchi, 1986, 1990) because of its mitotic instability, giving rise to several spontaneous sectors. From the Z mutant several improved and deteriorated sectors were isolated. The improved sectors presented colonies with normal morphology and conidiation, whereas the deteriorated (*det*) sectors presented slow growth rate, compact morphology, dark colour and sparse conidiation.

Figure 1 shows some characteristics of each sector, such as: normal, improved or deteriorated morphology; UV-sensitivity (*uvs*) or not (*uv*<sup>+</sup>); and level of mitotic instability.

Meiotic analyses of the crosses of the Z mutant and its 1st order sectors (Z1 and Z2) and 2nd order sectors derived from Z1 were made using the tester strain UT196. A control cross was made with UT448 x UT196. The results shown in Table I demonstrate a large variation in the meiotic *w-meth* recombination frequency, since the normal value is 1% (Clutterbuck, 1974, 1987).

Among the improved sectors (M), the recombination frequency in the *w-meth* interval was two to four times that of the control cross and similar to that found in the Z x UT196 cross. However, deteriorated sectors in the same interval showed recombination frequencies about 10 to 13 times higher than that of the control cross.

It is important to recall that deteriorated sectors are unstable and the improved ones are as stable as the normal strain. The data obtained show a close relationship between mitotic instability and alteration of recombination frequencies between *w-meth* markers. The same table also shows an apparent linkage of *meth* to chromosome I markers in all deteriorated (D) sectors (RF *meth-y* < 50%). Such

UT 448

N

I-0.0

$\omega^+$

M.N.N.G. treatment

Z

I-0.11

$\omega^+$

N - normal strain

Z - mutant

D - deteriorated sectors

M - improved sectors

I - mitotic instability (sectors/colony)

$\omega^+$  - UV sensitivity equal to that of the normal strain

$\omega^s$  - UV sensitivity higher than that of the normal strain

Z.1

D

I-0.24

$\omega^s$

Z.2

M

I-0.0

$\omega^+$

Z.1.1

M

I-0.01

$\omega^+$

Z.1.2

D

I-0.06

$\omega^s$

Z.1.3

D

I-0.14

$\omega^s$

Z.1.4

D

I-0.12

$\omega^s$

Z.1.2.1

M

I-0.0

$\omega^+$

Z.1.3.1

M

I-0.01

$\omega^+$

Z.1.4.1

M

I-0.0

$\omega^+$

Figure 1 - Origin of the spontaneous sectors and their main characteristics.

results could also suggest that there is a general reduction of recombination frequency but some of other experiments showed that this last possibility may be discarded.

In the improved sectors derived from the deteriorated ones, this apparent linkage was not evident suggesting that the loss of a *meth*<sup>+</sup> duplication restores stability and normal meiotic behavior. Therefore the presence of a tandem duplication was apparent through several anomalies present in the Z mutant, such as mitotic non-conformity and altered recombination frequencies (Figure 1 and Table I, respectively).

It is known that strains of *A. nidulans* bearing an extra-chromosomal segment show mitotic non-conformity but its stability can be restored after total or partial loss of the duplicate segment. This explanation suggests a possible error during DNA replication involving the loss of the duplicated (translocated) segment (Case and Roper, 1981) which can give rise to improved or deteriorated sectors. The latter could be explained by the occurrence of an *in tandem* duplication or by transposition of part of the extra-segment to another part of the genome (Azevedo and Roper, 1970).

For this reason and based on our data of Figure 1 and Table I, it is concluded that the Z mutant bears an *in tandem* duplication in chromosome II involving, at least, the *meth*<sup>+</sup> locus. Transposition of this duplicated element to chromosome I generates the Z1 deteriorated sector, and the Z2 improved sector is the result of total or partial loss of the duplicated segment of chromosome II.

Evidence for the transposition of *meth*<sup>+</sup> to chromosome I in the Z1 sector is shown in Table I, which shows that the transposed element includes *meth*<sup>+</sup> inserted in the *paba-y* interval of chromosome I.

The linkage of *meth* to *paba* and *y* in chromosome I and to *Acr* and *w* in chromosome II is evidence of this duplication in the Z1 derivative and its sectors. Improved sectors, derived from Z1, are the result of duplication loss from chromosome I.

The presence of *meth*<sup>+</sup> in chromosome I also promotes additional genetic alterations in the deteriorated sectors, close to the insertion point, which affect meiotic and mitotic behavior. These alterations may include genic rearrangements, that are quite similar to those resulting from transposon insertions (Nevers and Saedler, 1977).

Evidence of abnormal segregation of alleles in the neighbourhood of the apparent *meth*<sup>+</sup> insertion is presented in Table II, where the frequency of *w*<sup>+</sup> *meth*<sup>+</sup> recombinants [or *w*<sup>+</sup> *meth* (II)/*meth*<sup>+</sup> (I) pseudorecombinant classes] related to all segregant classes of *ribo-paba* and *paba-y* of chromosome I is given. Thus, it seems clear that the higher frequency of *meth*<sup>+</sup> *w*<sup>+</sup> recombinants is associated with a type of alteration existing in chromosome I, located in the *paba-y* interval of the deteriorated strains.

Table I - Meiotic recombination frequency (in %  $\pm$  S.D.) of meth marker (II) and *w*, *Acr* (II) and *paba*, *y*, *bi* (I) markers.

Marker	Control cross									
	196 x Z (247) (145)	196 x Z.2 (166) (M)	196 x Z.1 (212) (D)	196 x Z.1.1 (189) (M)	196 x Z.1.2 (114) (D)	196 x Z.1.3 (135) (D)	196 x Z.1.4 (270) (D)	196 x Z.1.2.1 (171) (M)	196 x Z.1.3.1 (244) (M)	196 x Z.1.4.1 (242) (M)
<i>w</i> -meth	2.7% $\pm$ 0.18	4.2% $\pm$ 0.28	27.8% $\pm$ 1.7	4.2% $\pm$ 0.26	18.4% $\pm$ 1.67	27.4% $\pm$ 2.3	34.4% $\pm$ 2.06	21.0% $\pm$ 1.56	6.9% $\pm$ 0.41	8.6% $\pm$ 0.52
<i>Acr</i> -meth	28.2% $\pm$ 2.29	26.5% $\pm$ 2.01	40.0% $\pm$ 2.7	20.1% $\pm$ 1.42	27.1% $\pm$ 2.49	28.1% $\pm$ 2.36	38.5% $\pm$ 2.31	28.6% $\pm$ 2.15	27.4% $\pm$ 1.72	23.5% $\pm$ 1.48
<i>paba</i> -meth	56.5% $\pm$ 7.49	47.5% $\pm$ 3.6	28.7% $\pm$ 1.93	47.6% $\pm$ 3.42	42.1% $\pm$ 3.88	37.0% $\pm$ 3.14	39.6% $\pm$ 2.38	59.6% $\pm$ 4.5	55.7% $\pm$ 3.53	55.3% $\pm$ 3.52
<i>y</i> -meth	50.5% $\pm$ 4.65	51.6% $\pm$ 3.9	26.0% $\pm$ 1.75	49.0% $\pm$ 3.53	26.0% $\pm$ 2.38	36.3% $\pm$ 3.08	30.1% $\pm$ 1.79	50.5% $\pm$ 3.82	56.2% $\pm$ 3.56	39.6% $\pm$ 2.51
<i>bi</i> -meth	48.2% $\pm$ 3.96	49.3% $\pm$ 3.78	33.0% $\pm$ 2.23	47.6% $\pm$ 3.42	40.3% $\pm$ 3.73	45.1% $\pm$ 3.83	44.0% $\pm$ 2.65	66.6% $\pm$ 5.05	63.1% $\pm$ 4.00	57.0% $\pm$ 3.63

(M) Improved sector; (D) deteriorated sector. The total number of colonies analyzed is given in parentheses.

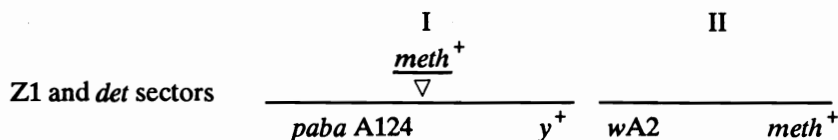
Table II -  $w^+ meth^+$  recombinants related to several intervals of chromosome I.

Chromosome intervals	Control 448 x 196 (145)	Z x 196 (247)	Z.1 x 196 (212) (D)	Z.1.1 x 196 (189) (M)	Z.1.2 x 196 (114) (D)	Z.1.3 x 196 (135) (D)	Z.1.4 x 196 (270) (D)
	$w^+ meth^+ \%$	$w^+ meth^+ \%$	$w^+ meth^+ \%$	$w^+ meth^+ \%$	$w^+ meth^+ \%$	$w^+ meth^+ \%$	$w^+ meth^+ \%$
<i>ribo paba</i>	0.7	0.4	12.1	1.0	6.0	13.2	15.8
<i>ribo<sup>+</sup> paba<sup>+</sup></i>	0.0	1.6	3.7	0.5	2.6	3.6	5.9
<i>ribo<sup>+</sup> paba</i>	1.3	2.0	9.8	1.0	8.6	6.6	8.8
<i>ribo paba<sup>+</sup></i>	0.0	0.0	1.8	0.5	0.8	3.6	3.2
<i>paba<sup>+</sup></i>	2.0	2.0	17.4	2.1	13.9	17.7	20.6
<i>paba<sup>+</sup> y</i>	0.0	1.6	2.3	1.0	2.6	5.8	7.0
<i>paba y</i>	0.0	0.4	4.6	0.0	0.8	2.1	4.0
<i>paba<sub>2</sub><sup>+</sup> y<sup>+</sup></i>	0.0	0.0	3.2	0.0	0.8	1.4	1.7

(D): deteriorated sector crossed to UT 196

(M): improved sector crossed to UT 196.

This alteration is the *meth*<sup>+</sup> duplication (*Dp* II-1) and the high number of *meth*<sup>+</sup>-*w*<sup>+</sup> recombinants results from the expression of a *meth*<sup>+</sup> transposed segment, that is:



This hypothesis fits well with our data and explains the recombination frequency of 27% between *w-meth* in the Z1 mutant. The RF expected for a *meth*<sup>+</sup> duplication is about 25% when such strains are crossed to a *w*<sup>+</sup> *meth* strain (UT196).

The dark colour and compact character of colonies and the *uvs* character of the deteriorated Z1 strain segregated more or less linked to *meth*<sup>+</sup> in a Mendelian fashion. These characteristics can be easily detected in the progeny.

The meiotic segregants were scored for UV sensitivity, with and without pre-incubation. The best results were obtained with 8 h of pre-incubation. The improved sectors and their meiotic segregants showed no UV sensitivity, whereas the deteriorated sectors and their meiotic segregants clearly showed a UV sensitivity (Table III).

The *uvs* character presented in all deteriorated sectors (Figure 1) may be explained by some alteration in DNA repair. The increased mitotic non-conformity of these mutants may be evidence of this observation. Majerfeld and Roper (1978) showed that chemical substances inhibiting DNA repair increase mitotic non-conformity and Jansen (1970a,b) found evidence for an association between *uvs* mutation and alteration of recombination frequencies.

The dark colour of the deteriorated colonies is an excellent genetic marker for detecting the presence of *meth*<sup>+</sup> in chromosome I as well as for detecting UV sensitivity. Since all the deteriorated colonies were UV sensitive this lead to the conclusions that the deterioration determinants and the *uvs* character are at the same *locus* or so closely linked that they segregate together.

Since meiotic crosses involving deteriorated sectors derived from Z1 (Table III) produced meiotic segregants sensitive to UV radiation it is possible to map the *uvs* character in linkage group I, close to the insertion point of the *meth*<sup>+</sup> duplication (Table IV).

The location of this *uvs* mutation is in agreement with that proposed by Jansen (1967) after mapping the *uvs* A1, but more studies are still needed to ascertain if they are allelic or not.

Table III - Meiotic segregation of the *uvs* character among the progeny of deteriorated (D) and improved sectors (M) crossed to UT 196.

	UT448 x UT196		Z x UT196		Z.1 (D) x UT196		Z.1.1 (M) x UT196	
	<i>uvs</i>	+	<i>uvs</i>	+	<i>uvs</i>	+	<i>uvs</i>	+
<i>ribo</i> <sup>+</sup>	0	77	0	130	40	75	0	94
<i>ribo</i>	0	68	0	117	58	39	0	95
<i>paba</i> <sup>+</sup>	0	75	0	119	10	101	0	90
<i>paba</i>	0	70	0	128	88	13	0	99
<i>y</i>	0	39	0	69	12	47	0	51
<i>y</i> <sup>+</sup>	0	46	0	81	38	22	0	53
<i>bi</i> <sup>+</sup>	0	71	0	118	18	82	0	86
<i>bi</i>	0	74	0	129	80	32	0	103
<i>Acr</i>	0	68	0	114	45	66	0	102
<i>Acr</i> <sup>+</sup>	0	77	0	133	53	48	0	87
<i>meth</i>	0	83	0	104	0	60	0	100
<i>meth</i> <sup>+</sup>	0	62	0	143	98	54	0	89
<i>w</i> <sup>+</sup>	0	85	0	150	50	69	0	104
<i>w</i>	0	60	0	97	48	45	0	85

Table IV - Location of the *uvs* marker.

UT196 x Z.1	
Interval	Recombination frequency
<i>ribo</i> - <i>uvs</i>	37.2%
<i>paba</i> - <i>uvs</i>	10.8%
<i>y</i> - <i>uvs</i>	28.5%
<i>bi</i> - <i>uvs</i>	23.5%
<i>Acr</i> - <i>uvs</i>	43.8%
<i>w</i> - <i>uvs</i>	44.8%
<i>meth</i> - <i>uvs</i>	25.4%

The behavior of the improved sectors of the Z1 strain are similar to others described in the literature (Zucchi and Azevedo, 1979; Case and Roper, 1984) since they result from the loss of the extra-translocated segments, and they restore mitotic stability, normal recombination frequencies and the  $uvr^+$  character except the Z1.2.1 (Table I) improved sector which may be the product of a non-efficient loss of the extra-segment.

The  $uvr$  character mapped by us is close to the break-point of *Dp* (I-II) found by Pritchard (1956) and by Nga and Roper (1968). Thus it is likely that the *paba-y* interval is important to originate translocations or insertion points or may even contain genes related to recombination control or DNA repair. In any case, the Z1 mutant and its sectors are significant since even through it bears a very small extra transposed segment, this mutant is relatively more stable than the strains bearing I-II (Azevedo, 1971) or III-VIII (Zucchi and Azevedo, 1979) duplications. In addition, the mutant is suitable for genetic studies establishing relationships between factors controlling local and general recombination. It was easy to construct heterozygous strains for the II-I duplication (Castro-Prado and Zucchi, in preparation).

## ACKNOWLEDGMENTS

The authors are grateful to CNPq-PIG (40.2534/82) and FAPESP (80/0737-8) for financial support.

Publication supported by FAPESP.

## RESUMO

Este estudo refere-se à descrição e caracterização de um mutante, induzido com MNNG que apresenta frequência de recombinação meiótica anormal no intervalo *w-meth* do cromossomo II de *Aspergillus nidulans*.

O mutante Z foi inicialmente selecionado devido ao seu caráter instável, dando origem a setores espontâneos, melhorados e deteriorados. Quando vários setores deteriorados foram analisados geneticamente, verificou-se que eles apresentaram instabilidade mitótica, alterações morfológicas, sensibilidade a luz ultra-violeta e um aparente aumento de recombinantes meióticos  $w^+ meth^+$ .

Todos estes aspectos foram interpretados como sendo a consequência da inserção de um segmento em duplicata, carregando o caráter  $meth^+$  do cromossomo II, para o intervalo *paba-y* do cromossomo I (*Dp* II-I). O caráter  $uvr$  e a morfologia deteriorada (*det*) são consequências diretas desta inserção.

O aumento substancial na frequência de recombinação *w-meth* está relacionado a expressão do gene  $meth^+$  no segmento transposto, uma vez que os setores melhorados espontâneos originados de

setores deteriorados, apresentam uma frequência de recombinação *w-meth* normal, morfologia normal e uma estabilidade mitótica completamente restaurada.

## REFERENCES

- Azevedo, J.L. (1971). Mitotic non-conformity in *Aspergillus nidulans*. Ph.D. Thesis, England University of Sheffield, 240 p.
- Azevedo, J.L. (1975). Altered instability due to genetic changes in a duplication strain of *Aspergillus nidulans*. *Gen. Res.* 26: 55-61.
- Azevedo, J.L. and Roper, J.A. (1970). Mitotic non conformity in *Aspergillus*: successive and transposable genetic changes. *Gen. Res.* 16: 79-93.
- Baimbridge, B.W. and Roper, J.A. (1966). Observations on the effects of a chromosome duplication in *Aspergillus nidulans*. *J. Gen. Microbiol.* 42: 417-424.
- Case, B.L. and Roper, J.A. (1981). Mitotic processes which restore genome balance in *Aspergillus nidulans*. *J. Gen. Microbiol.* 124: 9-16.
- Clutterbuck, A.J. (1974). *Aspergillus nidulans*. In: *Handbook of Genetics I* (King, R.C. ed.). Plenum Press, New York, pp. 447-510.
- Clutterbuck, A.J. (1981). Loci and linkage map of *Aspergillus nidulans*. *Aspergillus News Letters* 15: 58-72.
- Clutterbuck, A.J. (1987). Loci and linkage map of the filamentous fungus *Aspergillus nidulans* (O'Brien, S.L., ed.). Genetic maps. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Daud, F., Ortori, G.S. and Roper, J.A. (1985). Spontaneous I.R. duplications generated at mitosis in *Aspergillus nidulans*: further evidence of a preferential site of transposed attachment. *Genetics* 110: 229-245.
- Jansen, G.J.O. (1967). Some properties of the *uvs 1* mutant of *Aspergillus nidulans*. *Aspergillus Newsletter* 8: 20-21.
- Jansen, G.J.O. (1970a). Survival of *uvs B* and *uvs C* mutants of *Aspergillus nidulans* after UV irradiation. *Mutat. Res.* 10: 21-32.
- Jansen, G.J.O. (1970b). Abnormal frequencies of spontaneous mitotic recombination in *uvs B* and *uvs C* mutants of *Aspergillus nidulans*. *Mutat. Res.* 10: 33-41.
- Majerfeld, I.H. and Roper, J.A. (1978). The effects of coumarin on the frequency of deletions in a duplication strain of *Aspergillus nidulans*. *Mol. Gen. Genet.* 159: 203-206.
- Neves, P. and Saedler, H. (1977). Transposable genetic elements as agents of gene instability and chromosomal rearrangements. *Nature* 268: 109-115.
- Nga, B.H. and Roper, J.A. (1968). Quantitative intrachromosomal changes arising at mitosis in *Aspergillus nidulans*. *Genetics* 58: 193-209.
- Pontecorvo, G., Roper, J.A., Hemmons, L.M., MacDonald, K.D. and Bufton, A.W.J. (1953). The genetics of *Aspergillus nidulans*. *Adv. Genet.* 5: 141-238.
- Pritchard, R.H. (1956). A genetic investigation of some adenine requiring mutants of *Aspergillus nidulans*. Ph.D. Thesis, England, University of Glasgow.

- Van de Vate, C. and Jansen, G.J.O. (1978). Meiotic recombination in a duplication strain of *Aspergillus nidulans*. *Genet. Res.* 31: 29-52.
- 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 Odontologia - USP, Ribeirão Preto.
- Zucchi, T.M.A.D. and Azevedo, J.L. (1979). Mitotic instability in a III-VIII duplications strain of *Aspergillus nidulans*. *Rev. Bras. Genet.* 2: 93-108.
- Zucchi, T.M.A.D. (1990). Isolation of putative recombination mutants of *Aspergillus nidulans*. *Rev. Bras. Genet.* 13: 409-424.

(Received May 15, 1989)