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Atypical Trisomics In Aspergillus Nidulans

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ABSTRACT

The morphological specificity of the typical trisomic colonies in Aspergillus nidulans has been extended to atypical trisomics as well. The atypical trisomics were morphologically distinguished and genetically characterized. The specificity has been found to be the function of the extra homologue. Various mechanisms for the origin of the atypical trisomics have been postulated and in a few cases, it has been confirmed that the translocation of a segment of linkage group I to linkage group VI and subsequent mis-segregation of chromosomes led to morphological anomaly of the atypical trisomics.

Introduction

The mold *Aspergillus nidulans* is currently used as a test-organism in various fields of biological research, such as genetic transformation[1], recombinant DNA technology[2], molecular biology[3], cell biology[4], formal genetics[5] and environmental biotechnology[6]. The vegetative cycle of *Aspergillus nidulans* undergoes mitotic divisions only resulting in the production of the mitotic uninucleated cells. The conidia with the desired genetic constitution is synthesized in the laboratory, for exploring various genetic end-points. Another major advantage of *Aspergillus nidulans* is the availability of both the haploid and diploid stable strains. The stable diploids are formed in the para-sexual cycle[7], a process, which provides genetic variability even in the absence of meiosis. Through the occurrence of mitotic mis-segregation or, chromosomal mis-segregation[8] the spontaneous aneuploids are produced. These genetic events, either spontaneous or induced can be studied in detail in *Aspergillus nidulans* by genetic procedures, which are widely used in environmental mutagenesis[9] to identify genetically the chemicals, able to induce mitotic mis-segregation. The procedures are selective or non-selective depending upon the genetic makers used and offer different advantages[10]. The non-selective procedures are particularly suitable for detecting chromosomal mis-segregation, culminating in production of aneuploids, as morphologically unstable colonies. These colonies are usually less viable and competitive than euploid types and may not be recovered in the presence of partially inhibiting concentrations of selective agents[11].

The previous research communications from our laboratory dealt with the isolation, of spontaneous aneuploids in *Aspergillus nidulans* under liquid shake condition and the morphological and genetical characterization of the spontaneous typical disomics[12], typical trisomics[13], multiple trisomics[14] and non-segregating aneuploid colonies[15] in *Aspergillus nidulans*. In the present study the spontaneously emerging atypical trisomics have been isolated, spotted, morphologically identified and genetically characterized. An attempt has also been made to throw light on their mode of origin.

Materials and Methods

Strains : The heterozygous diploid strain of *Aspergillus nidulans* with markers on all the eight linkage groups was synthesized following Roper's technique[16] from the genetically well marked haploid strains of Glasgow stock (Fig. 1) and genetically analyzed. The general techniques and terminologies employed were those as described by Pontecorvo et al[17]. The liquid culture method[18] as suggested by Jha et al. (2009) was followed.

Fig. 1

Gene Symbols : Genes determining : conidial colour ; *y*A_2 = yellow , *w*A_3 = white, auxotrophic requirements ; *ad*E_{20} = adenine, *ribo* A_1 and *ribo* B_2 = riboflavin, *bi*A_1 = biotin, *phen* A_2 = phenylalanine, *pyro*A_4 = pyridoxine, *lys*B_5 =

lysine, *nic*B_8 = nicotinamide, inability to utilize a specific source ; *s*B_3 = thiosulphate, *gal*A_1 = galactose and *fac*A_{303} = acetate, resistance to chemicals; *Acr*A_1 = acriflavin, and suppressor; *su*$_1$*ad*E_{20} = suppressor of adenine requirement.
Treatment : The conidia of the heterozygous diploid strain was suspended in 0.876 per cent (N) saline with the detergent Tween – 80 at a final concentration of 0.1 per cent (v/v) . The conidial suspension was shaken vigorously on a Vortex mixer to break the chains of conidia. Approximately, 1 x 10^6 conidia were inoculated in 50ml liquid complete medium contained in a 250 ml flask. The flasks were shaken incubated on gyratory shaker at 37^0C for 3.5 hours and subsequently small samples of conidia were suitably diluted and plated at a low density on complete medium, so that only 10-12 colonies emerged on each plate. A random sample of slow-growing, sectoring colonies were spotted on incubation at 37^0C for 4-7 days. The conidia from their centers were collected from a few conidial heads by touching with a fine needle and rinsed off in 5 ml saline. The conidia from the suspension were plated on complete medium at various densities, with or without dilution, so that 10-12 colonies grew per plate. The genotypes of the spontaneously growing abnormals were determined by tracing out the segregation of markers in their stable sectors by the technique known as "sector analysis[19]". At least 28 sectors were tested for this purpose. In complicated cases, the number of sectors analyzed was higher.

Results and Discussion

The visually detectable, morphologically distinct, slow-growing, sectoring colonies were spotted and in each case, the detailed genetical investigations were done. All the aneuploids were found to be somatically unstable and usually produced euploid sectors. The markers in sectors always showed whole chromosome segregation. At least five kinds of aneuploids were found, e.g., typical disomics[12], typical trisomics[13], multiple trisomics[14], non-segregating aneuploid colonies[15] and atypical trisomics.

Aneuploids producing only diploid sectors and showing 2:1 segregation of markers located on the trisomic linkage group were grouped as typical trisomics[13]. At times, the ratio was subject to slight fluctuation either due to preferential isolation or variation in the viability and growth rate of the sector with respect to a particular genotype. The phenotypes of trisomics deviated only slightly in appearance from the corresponding disomic phenotypes, the former being larger in the size of the center of the aneuploid and bearing more conidiation. Like disomics, the trisomics also showed morphological specificity.

In addition to typical trisomics (2n+1), multiple trisomics[14] were also found. The multiple trisomics showed segregation of markers for two or more than two linkage groups. These also displayed morphological specificity, depending upon the involvement of linkage groups. Usually, the colonies were also visually detectable because of its intermediate morphology between the constituting ones. In general with the increasing number of additional linkage groups, the morphology of the colony exhibited greater anomaly, reduced colony size and poor conidiation.

The trisomic colonies producing sectors without segregation of markers were also observed. These colonies were identified on the basis of their gross phenotypic resemblance with the corresponding heterozygous trisomics. The non-segregation of markers in their euploid sectors could be explained on the assumption that in the (2n+1) condition, two of the trisomic chromosomes had the wild type alleles. Thus, its was visualized that phenotypic specificity existed irrespective of the difference in the genetic background of the homologue[15].

Morphologically, atypical trisomics resembled the typical trisomics and had the morphological characters of the typical trisomics, with which they tallied. For Illustration, the attention is confined to an atypical trisomic of linkage group VI only (Fig.2A & B).

Fig. 2

The morphological and genetical charactrization of the typical and atypical trisomic colonies for linkage group VI are summarized in Table 1. The centre of the typical trisomic colony for linkage group VI as a rule showed almost complete absence of conidiation, whereas the atypical trisomic for linkage group VI showed sparse conidiation in the centre. The colour of these typical and atypical trisomics were yellow and pale yellow respectively. The size of the colony as well as the size of the centre of the typical (2n+VI) were larger than corresponding ones. The shape of the centre of the typical trisomic was stellate and that of atypical ones circular. The genetical tests of the sectors

emanating from the typical trisomics showed that the ratio of segration was 2:1 (wild type : mutant maker) in case of typical trisomics but the atypical trisomics showed in some cases only wild type markers. The rate of the growth of the typical trisomic colony was faster than the atypical colonies.

Table 1

Table 1 Morphological and genetical characterization of the typical and atypical trisomic colonies

Features	Typical trisomic for linkage group VI	Atypical trisomic for linkage group VI
Conidiation in the center	Almost absent	Sparse
Colour of the Center	Yellow	Pale Yellow
Size of the center	Smaller (12±3 mm)	Larger (18±5 mm)
Size of the colony	Large (65±9 mm)	Small (50±5 mm)
Shape of the Center	Stellate	Almost Circular
Segregation ratio in the enploid sectors	2 : 1 (wild type : mutant marker)	2 : 1, and in some cases, the sectors showed only wild type markers
Rate of growth of the colony	Fast	Slow

The sectors arising out of the atypical trisomic colonies were also subjected to detailed genetical analyses. The diploid sectors emerging from the atypical trisomic colonies were mitotically haploidized on complete medium containing chloral hydrate (0.2M) and the segregation of markers in the resultant haploids were studied. It was found that in some of the cases, the segregation of the wild type and mutant markers located on linkage group VI did not obey 2:1 ratio of segregation for the markers located on linkage groups I and VI. In addition, the genetical analyses of the euploid sectors confirmed that the marker biA_1 of the linkage group I always segregated with the marker sB_3, located on linkage group VI, as if they were linked together (Table 2). Thus, it was concluded that atypical trisomic colonies also emerged, at least in a few cases, as a result of translocation of a segment of linkage group I to linkage VI, followed by mis-segregation of chromosomes.

Table 2 Detection of translocation by mitotic haploidisation of a diploid sector of an atypical trisomic colony on complete medium containing chloral hydrate (0.2M) by the analyses of the segregation of markers in the isolated haploid sectors.

Pairwise segregation of markers (trans)	Parentals		Recombinants		Recombination fraction
	+ +	- -	+ -	- +	
Acr A_1 ; bi A_1	29	20	18	35	53/102
Acr A_1 ; phen A_2	26	30	16	30	46/102
Acr A_1 ; pyro A_4	24	31	25	22	47/102
Acr A_1 ; lys B_5	18	26	30	28	58/102
Acr A_1 ; s B_3	29	20	18	35	53/102
Acr A_1 ; nic B_8	25	27	28	22	50/102
Acr A_1 ; ribo B_2	24	20	31	27	58/102
phen A_2 ; bi A_1	33	15	27	27	54/102
phen A_2 ; pyro A_4	24	23	24	31	55/102
phen A_2 ; lys B_5	18	34	21	29	50/102
phen A_2 ; s B_3	33	15	27	27	54/102
phen A_2 ; nic B_8	31	25	19	27	46/102
phen A_2 ; ribo B_2	21	27	23	31	54/102
pyro A_4 ; bi A_1	30	25	23	24	47/102

pyro A_4 ; lys B_5	30	21	21	30	51/102
pyro A_4 ; s B_3	30	25	23	24	47/102
pyro A_4 ; nic B_8	23	28	24	27	51/102
pyro A_4 ; ribo B_2	26	28	25	23	48/102
lys B_5 ; bi A_1	36	23	11	32	43/102
lys B_5 ; s B_3	36	23	11	32	43/102
lys B_5 ; nic B_8	35	25	20	22	42/102
lys B_5 ; ribo B_2	22	28	31	21	52/102
s B_3 ; bi A_1	49	53	00	00	00/102
s B_3 ; nic B_8	36	25	23	18	41/102
s B_3 ; ribo B_2	28	30	24	20	44/102
nic B_8 ; bi A_1	27	29	22	24	46/102
nic B_8 ; ribo B_2	33	29	27	13	40/102
ribo B_2 ; bi A_1	31	28	23	20	43/102

Pairwise segregation of markers (trans)	Parentals		Recombinants		Recombination fraction
	- +	+ -	- -	+ +	
y ; ribo A_1	27	26	24	25	49/102
y ; bi A_1	58	30	00	14	14/102

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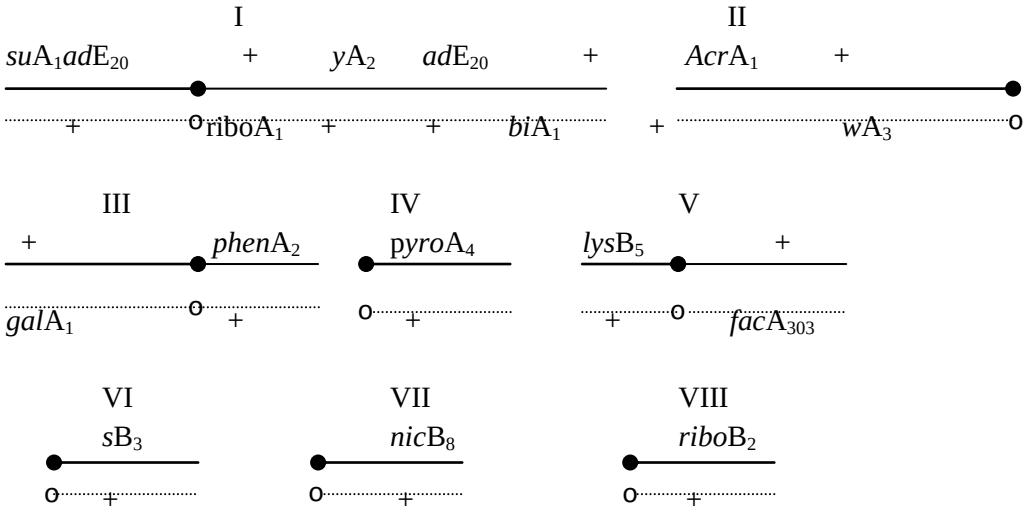
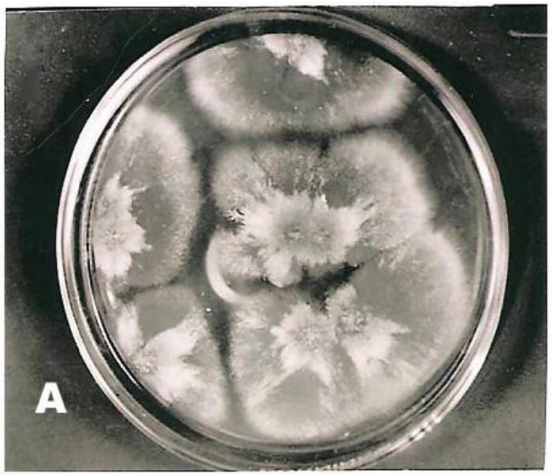


Figure 1. The genotype of the heterozygous diploid strain of *Aspergillus nidulans*.



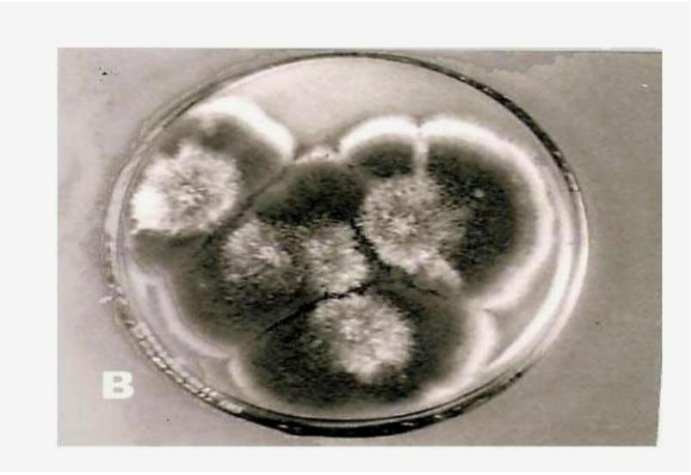


Figure 2. A. Typical trisomic colonies for linkage group VI.
B. Atypical trisomic colonies for linkage group VI.