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Characterization of an *Aspergillus nidulans* mutant with abnormal distribution of nuclei in hyphae, metulae, phialides and conidia

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Abstract

The V10 deteriorated variant of *Aspergillus nidulans* has hyphae, metulae, phialides and conidia with abnormal nuclear distributions. The alterations observed were: increase in the number of nuclei in hyphae, metulae and phialides, presence of anucleate, uninucleate and multinucleate conidia, abnormal vegetative growth and defective conidiation. When 0.5 M NaCl was added to the medium, an increase in the number of conidia was observed but their morphology and number of nuclei were not modified. The gene responsible for these alterations was named *anu*A1. The *anu*A1 gene is located on linkage group VII and is possibly involved in nuclear migration to hyphae, metulae, phialides and conidia.

Keywords: *Aspergillus nidulans*; Nuclear distribution; Conidial development

1. Introduction

The fungus *Aspergillus nidulans* presents parallel vegetative and sexual reproductive cycles. The germination of a vegetative haploid spore, the conidium, produces septated hyphae with multinucleate cells called conidiophores [14]. The vesicles are multinucleate and are formed at the conidiophore tips. A layer of metulae is formed on the surface of the vesicle and the phialides are produced from metulae by a single division of the nucleus. Metulae and phialides are referred to as sterigmata (primary and secondary, respectively). Conidia are formed by repeated mitotic division of the phialide nucleus. A spore wall is formed around the distal nucleus, which is arrested in the G1 phase of the cell cycle [14,18]. The metula, phialide and conidium are uninucleate.

Nuclear migration is very important for proper growth and development in both higher and lower eukaryotes. Anucleate primary sterigmata (*aps*) mutants of *A. nidulans* are partially blocked in conidiation due to failure of the organized migration of nuclei into the conidiophore metulae. The mutants fall into two complementation groups, *apsA* and *apsB*, mapping on chromosomes IV and VI, respectively [4–6]. Mutation in the *bncA* gene leads to binucleate conidiospores and abnormal distribution of nuclei in the metulae [13]. Another class of mutants with abnormal nuclear distribution was observed by Morris [9]. The gene symbol *nud* has been assigned to these mutants. The *nud*A, *nud*C and *nud*F genes were cloned and sequenced [11,20,21].

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Table 1
Number of conidia produced by *Aspergillus nidulans* strains AM and V10M grown on complete medium (CM) and complete medium with 0.5 M NaCl (CMNaCl)

Strains	Genotypes	Number of conidia ($\times 10^6$)[a]	
		CM	CMNaCl
AM	*pro*A1, *paba*A1, *y*A1	140.00	320.00
V10M	*pro*A1, *paba*A1, *y*A1; *anu*A1	2.10	16.00

[a]The number of conidia was estimated by transferring pieces of the colonies (three pieces of 0.6 cm diameter from each colony tested) to 2 ml 0.1% (v/v) Tween 80.

A. nidulans duplication strains produced sectors showing various degrees of phenotypic improvement and sectors with deteriorated morphology [2,10]. The improved sectors arose from nuclei with spontaneous deletions by an intrachromosomal process in either duplicate segment [2]. The deteriorated sectors analyzed have a linear growth rate usually about that of the parent duplication strains, reduced conidiation, and differently increased degrees of pigmentation. Different kinds of deteriorated variants were observed as follows: types with deletions from linkage group I; those with mutations in linkage group I or the II-I complex; and those with mutations elsewhere in the genome. Deteriorated strains are well suited to looking for conidiophore and conidia alterations.

In this paper nuclear distribution and conidiophore morphology were studied in deteriorated strain of *A. nidulans* denoted V10M. This mutant has a disordered nuclear distribution in the hyphae, metulae, phialides and conidia.

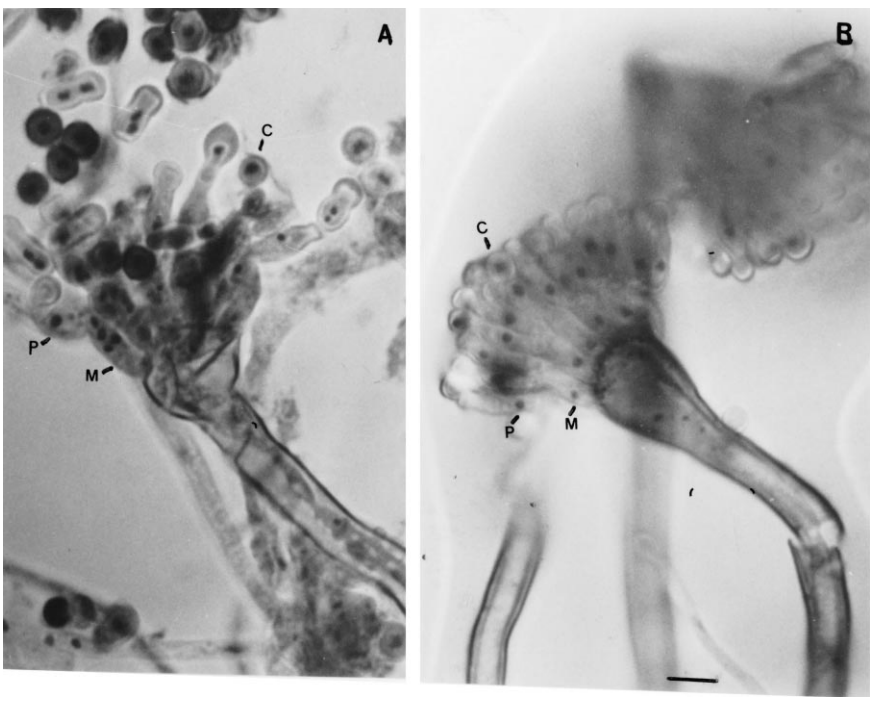

Fig. 1. Nuclear staining of conidia (C), metulae (M) and phialides (P) of *A. nidulans* V10M (A) and wild-type (B) strains with Giemsa-HCl. In the wild-type (B) all cells were uninucleate whereas strain V10M presented multinucleated metulae and conidia. Colonies were grown at 37°C on complete medium. Bar, 6.35 μm.

2. Material and methods

2.1. Strains and culture conditions

The strains of *A. nidulans* used in this work were from Glasgow stocks. The strains V3, V10 and V17 were isolated by Azevedo and Roper [2] as spontaneous sectors of the duplicated strains A, Dp(I-II) [10,15]. The strains AM and V10M were isolated as improved sectors of the strains A and V10, respectively. The strain V10W (*w*A1; *pyro*A4; *ribo*B2; *anu*A) was a segregant of the V10M × MSE cross. The MSE strain of *A. nidulans* [7] has markers in each chromosome (*su1ad*E20; *ad*E20; *w*A1; *gal*A1; *pyro*A4; *fac*A303; *s*B3; *nic*B8; *ribo*B2). Mutant alleles of importance in this work were the following: *y*A2 and *w*A1, white and yellow conidia, respectively; *fac*A303 and *gal*A1, unable to grow on acetate and galactose as the only source of carbon, respectively; *ad*E20, *nic*B8, *paba*A1, *pyro*A4, *pro*A1, *ribo*B2, *s*B3, requiring adenine, nicotinic acid, *p*-aminobenzoic acid, pyridoxine, proline, riboflavin and thiosulfate, respectively; and *su1ad*E20, a suppressor of *ad*E20. The minimal medium (MM) and complete medium (CM) used were described by Pontecorvo et al. [14]. The CMNaCl medium corresponded to CM with 0.5 M NaCl added.

2.2. Genetic analyses

Genetic analyses were performed as described by Pontecorvo et al. [14]. Diploids were prepared by Roper's [16] technique.

2.3. Growth rate and conidiation

Colony growth rates were measured with a ruler on the back of the dish after 6 days of incubation in CM and CMNaCl at 37°C. The number of conidia was estimated by transferring pieces of the colonies (three pieces of 0.6 cm diameter from each colony tested) to 2 ml 0.1% (v/v) Tween 80. Counts were made in a haemocytometer with four replicates per strain tested.

2.4. Nuclear staining and microscopy

Cultures for microscopic examination consisted of a film of complete medium with 10^6 conidia/ml on a coverslip incubated at 37°C for 19.5 h. Coverslips with adherent hyphae were transferred to methanol and incubated at room temperature for 5 min. The coverslips were incubated for 10 min at 63°C in 1 N HCl solution, rinsed in distilled water and transferred to 100 mM phosphate buffer (pH 7.0) prior to staining. For staining, the coverslips were incubated for 5 min at room temperature in a Giemsa solution [17] with 0.5 mg/ml Trypan Blue (INLAB). The coverslips were then rinsed in 100 mM phosphate buffer (pH 7.0) and mounted on clean glass slides and observed.

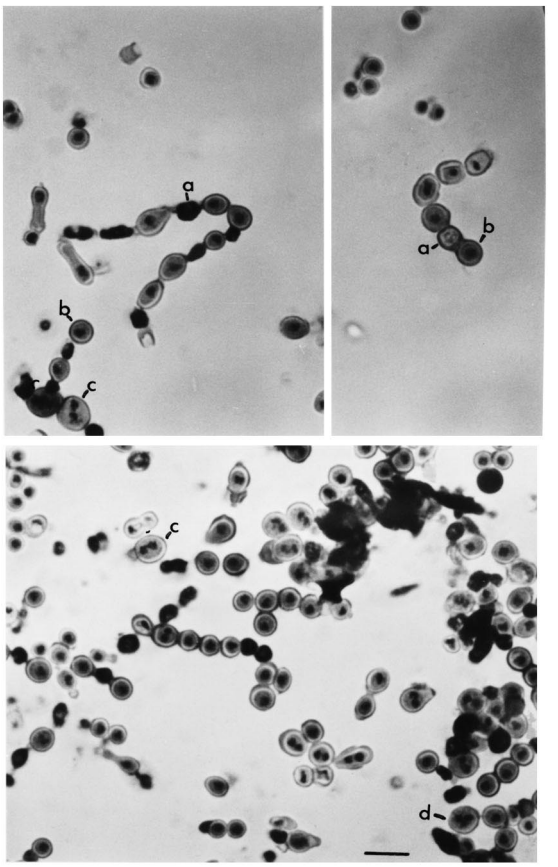

Fig. 2. Conidia nuclei from conidia of *A. nidulans* strain V10M stained with Giemsa-HCl. The conidia observed were heterogeneous with abnormal size and shape with zero (a), one (b), two (c) or three (d) nuclei. Colonies were grown at 37°C on complete medium. Bar, 6.35 μm.

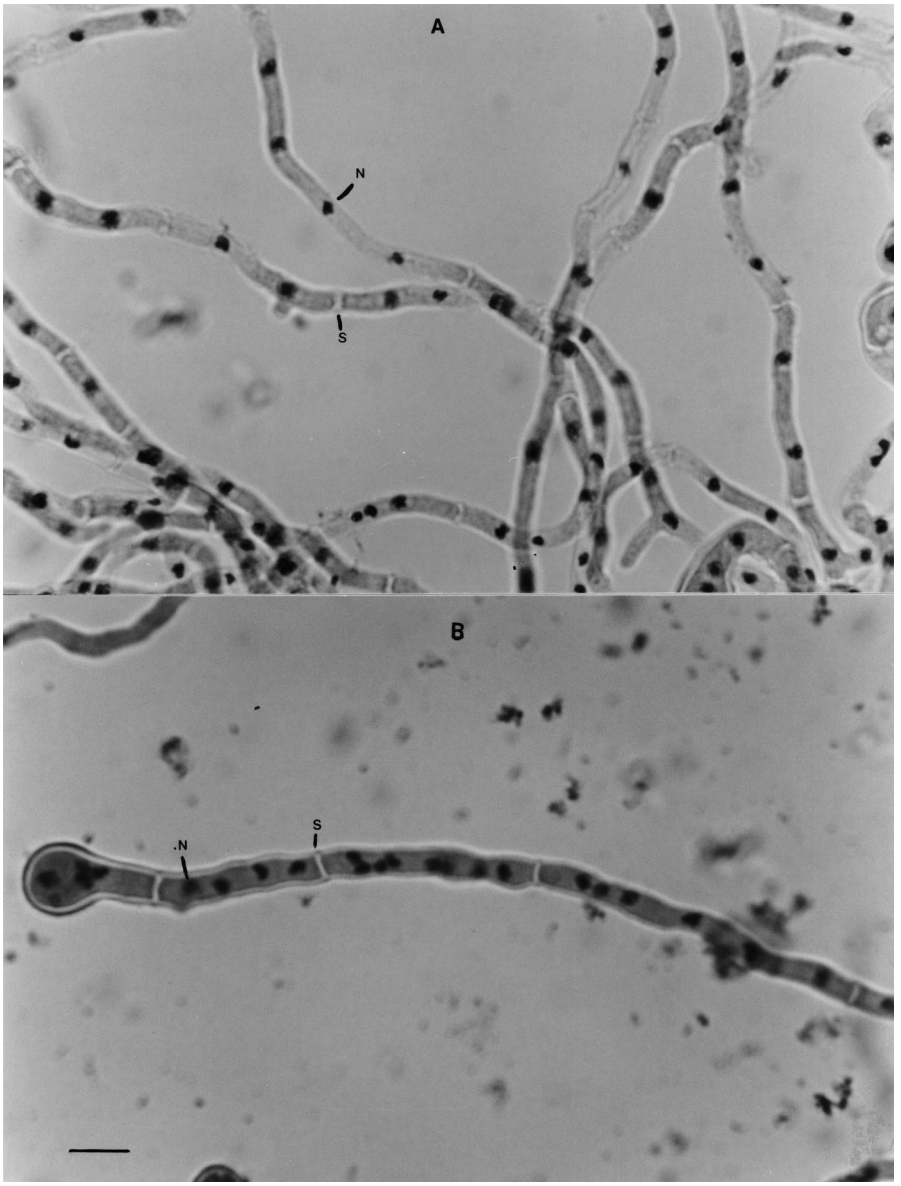

Fig. 3. Nuclear distribution in wild-type and V10M hyphae. The mycelia were grown on cover slips for 19.5 h. Nuclei (N) and septa (S) were visualized by Giemsa and Trypan Blue staining. A: Wild-type. B: V10M. Bar, 6.35 μm.

The conidial nuclei were stained with Giemsa based on the method of Tanaka et al. [17]. The coverslips were observed using an Olympus BHS microscope coupled with an Olympus PM-10AD photomicrography system, when necessary.

3. Results and discussion

This paper results from our interest in studying the morphological variations in deteriorated sectors spontaneously isolated from duplicated strains Dp(I-II) of *A. nidulans* [2,10]. The deteriorated variants are produced at a frequency of approximately

1 in ten colonies analyzed [1]. The deteriorated strains show poor conidiation and different degrees of pigmentation. Three variants isolated by Azevedo and Roper [2] were chosen for analysis (V3, V10 and V17). The deteriorated variant V10 was the only one that showed morphological alterations in the conidia, metulae and phialides. To eliminate duplication interference an improved sector of V10, designated V10M, was isolated. This strain had brown mycelia and poor conidiation when grown on CM. When 0.5 M NaCl was added to CM, the conidiation rate was increased, but it was not similar to the rate of sporulation of the AM strain grown in the same conditions (Table 1).

The yield of conidia from strains containing multiple genetic lesions that conidiate poorly can often be increased by incorporating high levels of supplements into the growth medium and by increasing the osmotic pressure of the medium through the addition of 1.2 M D-sorbitol or 0.7 M KCl [19]. In the presence of 0.6 M KCl, four deteriorated variants isolated as sectors from duplicated strains of *A. nidulans* displayed normal morphology [8]. The deteriorated variant V10M showed a 7.6-fold increase in the rate of sporulation in the presence of 0.5 M NaCl compared to a 2.3-fold increase for AM. However, this increase of conidiation in the presence of the salt was not sufficient to consider V10M as a phenocopy of the AM strain, since V10M continued to have morphological alterations.

The observation of conidia, metulae and phialides of the V10M variant by microscopy showed a number of differences from the wild-type (Figs. 1 and 2). The distribution of nuclei in hyphal compartments is investigated in Figs. 3 and 4. V10M shows 83% of the compartments containing more than two nuclei compared to 35% in the wild-type. The irregular distribution of nuclei in vegetative hyphae was observed by Clutterbuck [5] in the *aps*A6 mutant of *A. nidulans*. All the *aps* mutants examined exhibited high concentrations of nuclei in hyphal tip cells with distribution of nuclei in clusters separated by conspicuous gaps.

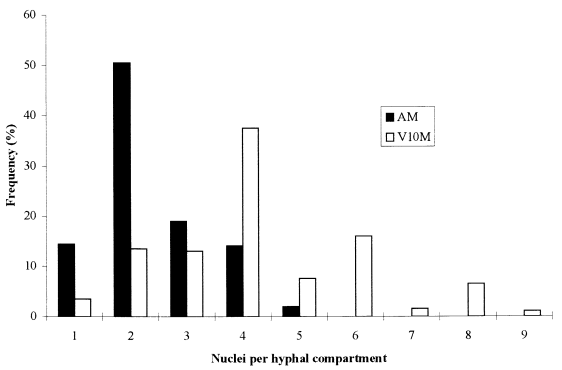

Fig. 4. Frequencies of hyphal compartments containing different numbers of nuclei in wild-type and V10M strains. Nuclei from 60 compartments were counted.

The metulae and phialides observed in V10M were long and swollen (Fig. 1). Some of the conidia of V10M variants were large, oval or shaped in the form of small bottles (Figs. 1 and 2). About 1–3 nuclei were present per metula, phialide or conidium and anucleate conidia were also observed. Table 2 shows the number of anucleate, uninucleate and multinucleate conidia found in V10M compared to other strains, a heterokaryon and a diploid between V10M and MSE strains. The anucleate conidia were often in an intermediate position in the chain of spores and sometimes black conidia were observed, possibly resulting from degeneration of anucleate conidia (Fig. 2). Normally, after the developing conidium had received one nucleus and reached about 3 μm in diameter a septum formed separating the conidium from the phialide. However, it was possible to see phialides completely connected with conidia without the presence of a septum and two conidia united in the V10M variant, as if the spore separation step had failed (Figs. 1 and 2).

A heterokaryon between strains V10M and MSE, which has genetic markers in each chromosome of *A. nidulans*, also showed anucleate and multinucleate conidia, but at a rate lower than that of the V10M variant (Table 2). The cleistothecia produced from the heterokaryon between strains V10M and V10W were examined and of the 60 analyzed none showed ascospore production. The mutant *aps*A18 analyzed by Clutterbuck [5] was also sexually sterile but all other *aps*A mutants appeared normal. Diploid strain contained uninucleate, binucleate and anucleate conidia (results not shown). After analysis of nuclear distribution in hyphae, conidia, metulae and phialides of the V10M mutant, the gene responsible for this phenotype was named *anu*A1 (anucleate). The gene *anu*A was mapped by the parasexual methods

Table 2
Number of nuclei present in the conidia of the AM, V10M, MSE, heterocaryon (V10+MSE) and diploid (V10M/MSE) strains of *Aspergillus nidulans*

Strains	Number of nuclei (%)			
	0	1	2	3
AM	0 (0)	400 (100)	0 (0)	0 (0)
V10M	65 (16)	276 (69)	54 (14)	5 (1)
MSE	0 (0)	400 (100)	0 (0)	0 (0)
V10M+MSE	30 (7.5)	360 (90)	10 (2.5)	0 (0)
V10M/MSE	6 (1.5)	388 (97)	6 (1.5)	0 (0)

to chromosome VII (results not shown) and is evidently the same gene as that formerly designated *v*10 by Azevedo and Roper [2] as conferring a mutant V10 deteriorated phenotype. A diploid strain homozygous for *anu*A1 was very unstable, sometimes produced sectors and appeared to be a heterokaryon.

Mutants defective in nuclear migration have been described and studied in *A. nidulans* [3–6,9,11–13]. The genes *anu*A1 and *bnc*A1 [5,13] share similar characteristics such as the presence of multinucleate conidia and alterations in the metulae and phialides. However, the mutation in *bnc*A did not produce anucleate conidia and *bnc*A was mapped to a different chromosome [13].

This paper represents the first step in the study of a gene of *A. nidulans* possibly involved in nuclear migration of hyphae, metulae, phialides and conidia.

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