CYTOLOGICAL CHARACTERIZATION OF AN *ASPERGILLUS NIDULANS* MUTANT FROM A STRAIN WITH CHROMOSOMIC DUPLICATION

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ABSTRACT

A development mutant, named V103, was obtained spontaneously from the A strain of *A. nidulans*. The A strain contains a duplicated segment of chromosome I that has undergone translocation to chromosome II (I → II). It is mitotically unstable and generates phenotypically deteriorated types, some with enhanced stability. The deteriorated variants of *A. nidulans* show abnormal development, exhibiting slower colony growth, variations in colony pigmentation and changes in conidiophore structure. The alterations observed in the conidiophore include fewer metulae and phialides, further elongation and ramification of these structures, delayed nuclear migration and the presence of secondary conidiophores.

Key words: *Aspergillus nidulans*, Conidiogenesis, Nuclear migration, Asexual Cycle, Mitotic Instability

INTRODUCTION

The life cycle of the fungus *Aspergillus nidulans* is marked by important developmental events, which include the germination of asexual, uninucleate mitotic spores (conidia), nuclear migration through the mycelium and the establishment of a highly polarized growth pattern that gives rise to multinucleate hyphae (6). Nuclear migration is an essential feature for the growth of filamentous fungi and the process of conidiation involves temporal and special regulation of gene expression, cell specialization, and intercellular communication (1). In this context, several classes of mutants with abnormal nuclear distribution that impairs hyphal extension have been observed. For instance, nuclear distribution (*nud)* mutants contain nuclei that divide at a normal rate but fail to move from the spore end of the growing germ tube (12, 13). In *nud* mutants, gene products essential for nuclear distribution in hyphae have lost function, resulting in abnormal conidiophore morphology (24). Furthermore, in *bim* mutants (blocked in mitosis – cell cycle mutants), a wide range of cell cycle functions involved in mitotic spindle formation are impaired (4, 8).

The germination of conidia produces septated hyphae with conidiophores (17). The vesicles are multinucleate and develop at the conidiophore tips. A layer of metulae forms on the surface of the vesicle and the metulae produce phialides by a single division of nucleus. Repeated mitotic division of the phialide nucleus generates conidia. (10, 21). A limited set of regulatory loci controls the coordinates temporal and special expression of hundreds of genes required for morphogenesis. The sequential activation of three of these genes, *bristle (brlA), abacus (abaA)*

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and *wet-white (wetA)*, establishes the central regulatory pathway for control of gene expression observed during conidiophore development (5). Two other genes, *stuA* and *medA*, interact with key regulatory genes and may be considered morphological modifiers. Mutants for these genes are oligosporous because of abnormalities in their conidiophores (1).

In this study, we have characterized a new, spontaneously obtained mutant of *Aspergillus nidulans* with substantial developmental changes. We investigated conidiophore development and the cell cycle of V103, a strain derived from the A strain of *A. nidulans,* which is mitotically unstable because of a partial chromosomal duplication followed by translocation (I → II). We have shown that this development mutant exhibits significant changes in colony growth, colony pigmentation and conidiophore structure. In V103, the conidiophore contained fewer metulae and phialides, with further elongation and ramification of these structures. We have also observed delayed nuclear migration and the presence of secondary conidiophores.

MATERIALS AND METHODS

Strains

The haploid strains of *A. nidulans* were derived from Glasgow stocks. For this study, the deteriorated strain V103 was isolated from spontaneous sectors of the duplicated A strain, Dp (I-II). The MSE strain of *A. nidulans* (9) has the following markers in each chromosome: wA_3 (II), $facA_{303}$ (V), $galA_1$ (III), yA_1 (I), $pyroA_4$ (IV), sB_3 (VI), $nicB_6$ (VII) and $riboB_2$ (VIII). The A strain of *A. nidulans* (14, 15) has the markers $proA_1$ (I), $pabaA_6$ (I) and Dp (I-II).

Culture medium and Conidiogenesis

Complete medium (CM) (17).

Conidia from the V103 strain were incubated in liquid culture for 2, 4, 6, 8, 12, 16 and 20 hours at 37°C. Synchronous conidiophore development was induced by transferring a thin mycelial at filtered from liquid culture to agar plates (Agar

Complete Medium) with inserted coverslips and inoculating the plates for 16, 20, 24, 32, 48 and 72 hours at 37°C.

DAPI and Calcofluor staining

The deteriorated V103 variant was removed from the growth medium at different times and immediately fixed in a solution of 3.7% formaldehyde, 50 mM Na_2HPO_4 (pH 7.0) and 0.2% Tween 80 for 30 minutes. Samples were washed in water and stained for 5 minutes with 0.1 μg/ml 4´, 6-diamidino-2-phenylindole (DAPI, Sigma) and 0.4 μg/ml of Calcofluor (Sigma)(6). After one wash in distilled water, the slides were mounted in Vectashield medium (Vector). The preparations were examined under UV light, using an Axioplan 2 - Zeiss microscope.

Scanning Electron Microscopy (SEM)

Cultures for Scanning Electron Microscopy examination consisted of coverslips with adherent hyphae (Agar Complete Medium). The conidiophores were transferred to a solution containing 25% glutaraldehyde, 10% paraformaldehyde in cacodylate buffer (0.2 M, pH 7.2) and $CaCl_2$ (0.1 M). After 1 – 2 hours the coverslips were washed for 10 minutes with buffer and post fixed for 1 hour in similarly buffered OsO_4 (1%). The coverslips were then washed in distilled water and dehydrated in a graded acetone series. The coverslips were critical point-dried (Balzers CPD 030), coated with gold (MED 010 - Balzers), and examined with a Scanning Electron Microscope (Leo 435 VP).

RESULTS AND DISCUSSION

This work results from an interest in studying the cellular cycle and conidiophore development in a deteriorated sector spontaneously isolated from the Dp (I-II) duplication strain of *A. nidulans,* (2, 3, 18). In this strain, a segment of chromosome I was duplicated and translocated to chromosome II. The translocated segment was mapped by the sexual and parasexual recombination methods to chromosome I and is located 15.38 cM away from the marker $proA_1$, $pabaA_6$ of the MSE strain (data not shown).

We investigated the nuclear division cycle during conidial germination to determine if the V103 mutation affected this stage of the *A. nidulans* life cycle. Conidial suspensions of V103 were inoculated onto glass coverslips. Samples were collected at several time points and the nuclei were visualized using DAPI and Calcofluor staining. Figure 1 shows the wild type (MSE) and V103 germlings at different stages of development. In the V103 strain, germ tubes are shorter, but appear to establish a normal growth polarity. In contrast to the wild type, nuclei in the mutant strain are randomly distributed along the germ tube, suggesting a disruption in nuclear distribution and positioning. The first septum in the wild type germling forms shortly after the third nuclear division. This septum is deposited at the basal end of the longer germ tube (22). In this regard, the V103 mutant was similar to the wild type, but approximately 40% of the germlings had a septum at the basal end of the shorter germ tube, suggesting a dysfunction in the mechanisms that coordinate polar growth and septation. The V103 mutation generates phenotypes similar to those observed in several previously classified mutations that affect the cell duplication cycle. For instance, similar phenotypes were observed in *blocked in mitosis* (*bim*) mutants (12, 13). The *bim* mutation affects a wide range of cell cycle functions (8). Mutants identified as defective in septation also have some characteristics in common with the V103 mutant, such as increased numbers of nuclei per hyphal compartment, the occurrence of mitotic catastrophe and abnormal growth polarity (12). V103 showed aberrant morphology similar to that of the *sep* mutant, resulting in growth polarity abnormalities and deposition of the actin ring during cytokinesis. In turn, this may lead to an increased chromosome mitotic index (1). In addition, analysis of *hyp* phenotype suggests that wild type *hyp*A promotes tip growth and restrains growth of basal cells (7, 20).
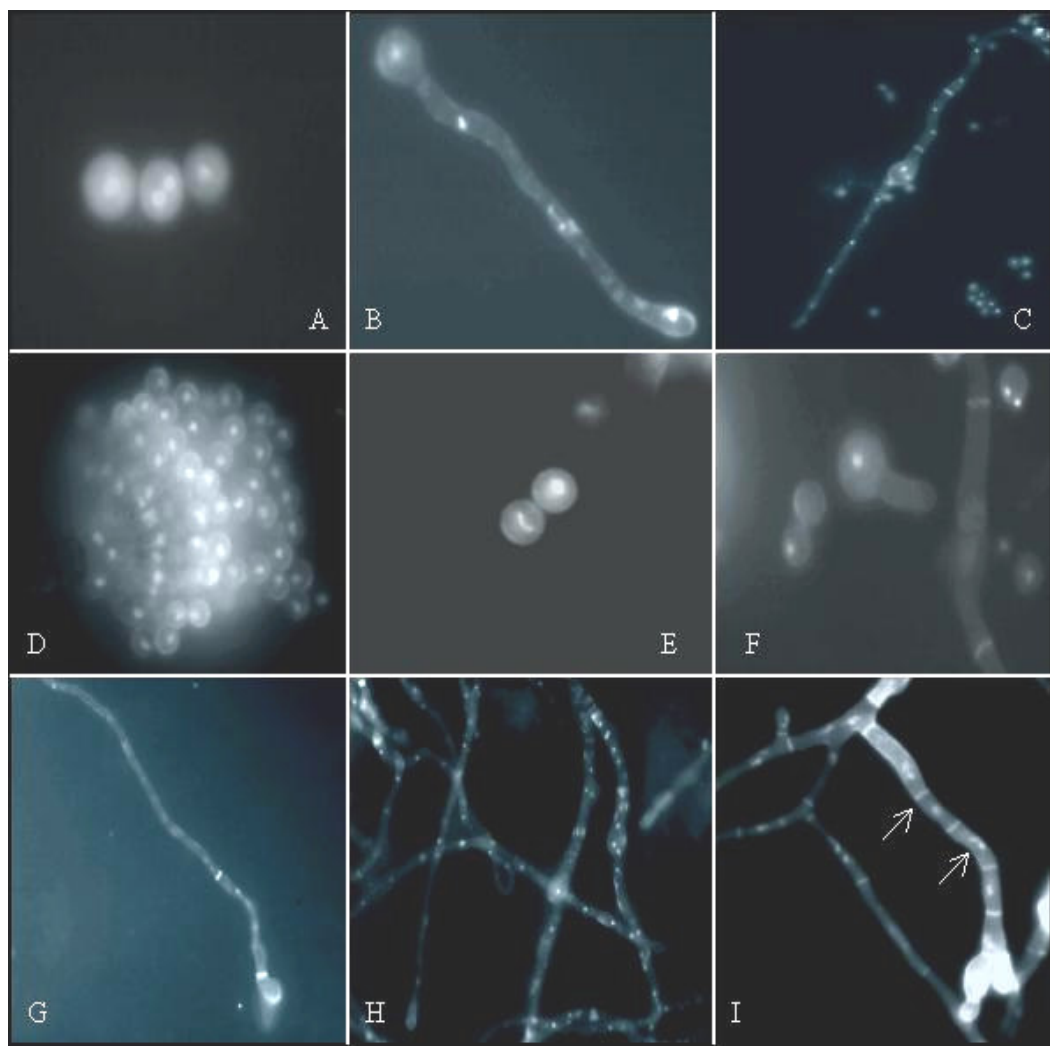

Figure 1. DAPI and Calcofluor staining (Agar Complete Medium). A–D: wild-type strain at 2-4, 6-8, 12-16 and 32 hours respectively. E–I: the V103 deteriorated variant at 2-4, 6-8, 12-16, 20-24 and 32 hours respectively. H: Defective growth of the septes, I: Septation and multiple nuclei in the conidiophore stalk (arrows). Magnification: C 400× and A – I 1000×.

In both the MSE and V103 strains of *A. nidulans*, conidiation began with formation of the aerial hyphae that constitute the conidiophore stalk. The stalk arises as a vertical outgrowth from a hyphal compartment known as the foot cell. The stalk tip swells to form the conidiophore vesicle. Numerous small outgrowths termed metulae develop from this vesicle. Metulae give rise to sporogenous cells, termed phialides. One phialide forms at the tip of each metulae and additional phialides appear below and to the sides of the first phialide. The phialides begin to form asexual spores termed conidia that accumulate in chains at the ends of the phialides, as shown in Figure 2. (10, 16). The metulae and phialides observed in V103 were long and indistinct, presented secondary conidiophores, multiple tiers of sterigmata (metulae and phialides) and conidiophore stalks with many septa were apparent. In the wild type, usually a single vesicle is formed followed by a single tier of metulae and a single tier of phialides. In contrast, in V103's the conidiophore stalks ramified and formed secondary conidiophores (Figure 2).
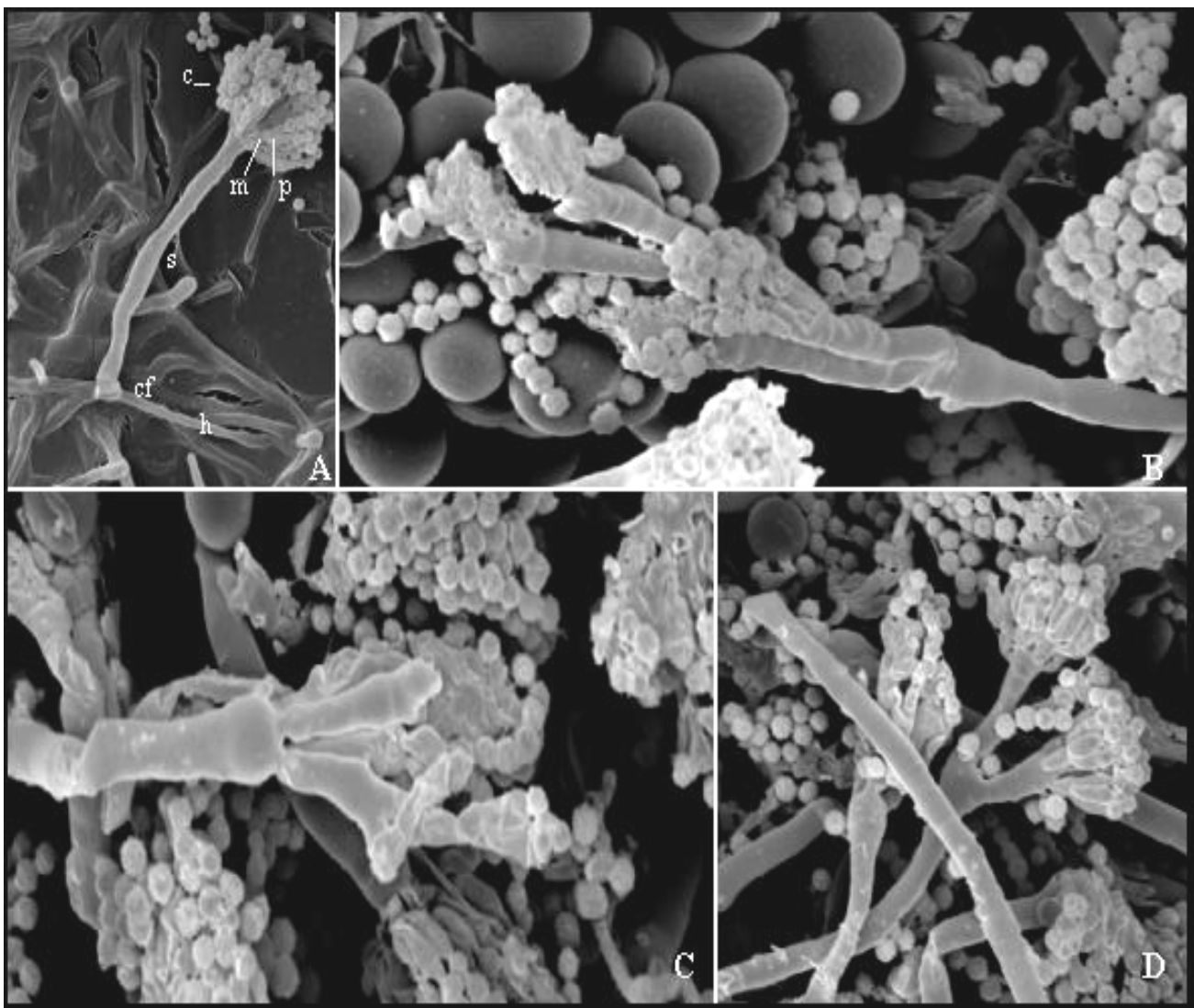

Figure 2. SEM showing conidia (c), metulae (m), phialides (p), stalk (s), foot-cell (cf) and hyphae (h) of wild-type (A) and V103 (B, C and D) strains of *A. nidulans*. In B and C, metulae and phialides were long, indistinct and showed secondary conidiophores. In D, Conidiophore stalks ramify. Colonies were grown at 37 °C in Agar Complete Medium for 48 hours . Magnification from A to D: 2500×, 3030×, 3420× and 3490×, respectively.

Cytological characterization of the cell division cycle, nuclear movement and developmental morphogenesis of the asexual development *A. nidulans* indicates that a complex regulatory system is at work. This system is mainly characterized by transcriptional control mechanisms leading to differential expression of structural genes required for conidiophore formation. We reported, cytologically, that the pattern of cell growth, nuclear division, and cytokinesis changes dramatically during formation of the different conidiophores cells. It has been proposed that the cell cycle and the developmental program may interact (11). Ye *et al.* (23, 24) have shown that the main cell cycle regulators NIMX^{cdc2} and NIMA are upregulated on mRNA and kinase activity levels in a *brl*A-dependent manner. Schier *et al.* (19) have isolated the cyclin *pcl*A developmental gene required for the fast, repetitive cell divisions of the phialides, which subsequently lead to the long conidial chains of the conidiophore. This gene mediates events of the cell cycle and developmental morphogenesis

This paper represents the first analysis of the development of a spontaneously obtained mutant, starting from an inserted segment, shown the need the molecular analyses to deepen the knowledge and importance among the interactions of the cell cycle with the regulator genes of the conidiogeneses.

ACKNOWLEDGMENTS

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