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Phenotypic characterization of the morphological mutant UVM9 of *Sporothrix schenckii*

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ABSTRACT. Dimorphic transition of some fungal pathogens is an important attribute of some pathogenic fungi. The human pathogen *Sporothrix schenckii* is a dimorphic fungus, capable of growth either as filamentous hyphae or as yeast budding cells in response to environmental conditions. In the present study we report the phenotypic characteristics of the morphological mutant UVM9, obtained from a clinical isolate of *Sporothrix schenckii*. Mutant strain could not develop as budding cell, though it switches to hyphae cells shape at any culture condition. However the polarized growth and extension of the germ tube could not be sustained and abnormal short and thick hypha with atypical conidia-like cells were observed. Resistance phenotype to drugs, which target cytoskeleton and cell wall suggest that the integrity of these structures is altered in the mutant. Pleiotropic phenotype could be explained by an alteration in the tubulin cytoskeleton.

Key words: *Sporothrix schenckii*, dimorphism, pathogenic fungus, morphological mutant, cytoskeleton.

RESUMEN. La transición dimórfica es una característica importante de algunos hongos patógenos que tienen la capacidad de crecer como células filamentosas (hifas) o como células gemantes (levaduras) en respuesta a cambios del medio. *Sporothrix schenckii* es el hongo causante de la esporotricosis, micosis subcutánea frecuente en climas templados. Este microorganismo es dimórfico y crece como hifas y conidios en su forma saprobia y se diferencia a levadura dentro del huésped. *In vitro* los cambios morfológicos son inducidos a diferentes pHs del medio: a pH 5.5 crece como hifas y produce conidios piriformes y a pH 7.2 crece como levaduras gemantes. Durante el proceso de dimorfismo se llevan a cabo cambios importantes en la polaridad del crecimiento celular que involucran cambios en el citoesqueleto y en la pared celular. En este estudio nosotros reportamos las características fenotípicas de una mutante morfológica de *S. schenckii* (UVM9). La mutante no es capaz de responder a los cambios de pH del medio de cultivo y en cualquier condición crece como hifas cortas y gruesas que producen conidios atípicos. El fenotipo de resistencia a diferentes fármacos que tienen como blanco al citoesqueleto mostró que estas estructuras están alteradas en la mutante. Este efecto pleiotrópico puede ser explicado como resultado de una alteración en el citoesqueleto de tubulina.

Palabras clave: *Sporothrix schenckii*, dimorfismo, hongo patógeno, mutante morfológica, citoesqueleto.

INTRODUCTION

The dimorphic fungi *Sporothrix schenckii* is the causal agent of sporothrichosis, a chronic human disease. The traumatic inoculation of hyphae and conidia causes a subcutaneous mycosis; the fungus differentiates to yeast form and may spread to other tissues [Kwon-Chung and Bennett, 1992; Travassos, 1985]. The mechanisms that control germination and growth as a yeast or hyphae in *S. schenckii* are not known. Though it has been reported for different fungi, that alterations in regulatory genes as well as in structural components of cell wall, and the cytoskeleton, will alter the cells shape and colony morphology [Alison *et al*., 1984; Madhani and Fink, 1998; Xiang *et al*., 2003].

Fungal cell morphogenesis (as yeast or filament) depends on polarization of the cytoskeleton and other components of the morphogenetic machinery needed for the assemblage of secretory vesicles intimately linked to cell growth [Akashi *et al*., 1994; Seiler *et al*., 1997; Wu *et al*., 1998; Schuchardt *et al*., 2005]. Filamentous fungi have a brief period of isotropic expansion and switch to a germ tube with apical extension. New material is added exclusively to the apex with branches that emerge from the main hyphae. Yeast starts with a period of isotropic expansion that later switches to polar growth to allow bud emergence [Harris and Momany, 2004].

In vitro, *S. schenckii* cells are able to respond to a large variety of environmental signals (e.g. temperature, pH, and

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culture medium) that promote the development of yeast cells, or as long branching filaments where conidia grow laterally on the hyphae or in clusters in the conidiophores [Kwon-Chung and Bennett, 1992; Travassos, 1985]. Conidia have a brief period of isotropic expansion, further can switch to a germ tube with apical extension for hyphae development or it can continue the isotropic expansion during yeast development and switch to polar growth to allow bud emergence [Travassos, 1985; Resto and Rodríguez del Valle, 1983]. The study of *S. schenckii* morphological processes has been hampered since genetics, molecular transformation, and targeting of specific genes have failed to fulfill this objective. To start our studies in *S. schenckii* morphogenesis we took advantage of the high frequency and variable phenotypes of morphological mutants obtained after UV light exposure. From the different phenotypes described, the UVM9 mutant was selected with the following criteria: i) it has pleiotropic traits as altered shape and size, ii) is a stable mutant with a reversion frequency of 1 x 10^{-5} [Torres-Guerrero *et al*., 1998] suggesting that the phenotype is the result of the mutation is in one gene and is not the result of multiple events and iii) it is avirulent in a murine model. Mice infected with the mutant exhibited 95% survival compared to 10% survival with wild-type.

In this work, we analyzed the behavior of the mutant during dimorphic transition induced by the pH of the medium. The results show that the conidia could not develop into budding cells at pH 7.2 but it could grow at pH 5.5 as germ tube and hyphae. The morphology and polarized growth of the hyphae were severely altered and short and thick hyphae with limited growth were produced. The conidia developed from these hyphae were round compared with the pyriform morphology of the wild-type.

The phenotypic characterization of resistance to benomyl, griseofulvin, and calcofluor entail that the structure of cytoskeleton and cell wall of the mutant are altered. Our results suggest that apical extension is not maintained probably due to alterations in cytoskeleton.

METHODS

Strains and growth conditions

Wild-type strain FM217 was isolated from a patient with sporotrichosis and was kindly provided by Jorge Mayorga, Instituto Dermatológico, Jalisco, México. UVM9 is a morphological mutant, isogenic to FM217 [Torres-Guerrero *et al*., 1998]. Conidial suspension was prepared by adding sterile water to YEPD slants (0.1% w/v glucose, 0.3% w/v peptone, and 0.2% w/v yeast extract) that were incubated for 7 days at 28 ºC. This suspension was filtered (sterile Whatman paper No 1) to selectively eliminate short hyphae. To obtain yeast and hyphae shapes, 1 x 10^7 conidia ml^{-1} were inoculated in basal medium with glucose and vitamins (RV) as previously described [Rodríguez del Valle *et al*., 1983; Torres-Guerrero *et al*., 1998]. In RV pH 7.2 conidia developed as yeast cells while mycelium was observed at pH 5.5. Conidia were also grown in liquid or solid YEPD at 28 ºC to induce mycelium. At 12, 18, and 24 h of incubation, samples were taken and fixed with 2% formaldehyde. Cells were observed by Differential Interference Contrast DIC (Olympus Provis).

Inhibitory drug concentrations (IC_{50})

Conida from YEPD slants cultured for 7 days were washed with water, and the suspension was adjusted to 1 x 10^6 cells ml^{-1}. Appropriate dilutions of cells were spread on YEPD containing the different drug concentrations: cytochalasin B (3-5 µg ml^{-1}), benomyl (0.2-1 µg ml^{-1} (methyl 1-butylcarbamoyl)-2-benzimidazolecarbamate), griseofulvin (0.5-1 mg ml^{-1}), and calcofluor (2.5-12.5 µg ml^{-1}) (fluorescent brightener 28) were purchased from Sigma (Sigma Chemical Co., St Louis, Mo). Colonies were counted after 7 days of incubation at 28 ºC. Percent of survival was determined by comparing the number of colonies on plates with the drugs to those on plates without drugs. The experiments were repeated independently 5 times with both strains.

Lyticase sensitivity assays

Conidia and yeast cells from the wild-type and conidia from the UVM9 strain were suspended in spheroplasting buffer (1.2M sorbitol, 10 mM potassium phosphate, pH 7.2) at a final concentration of 1 x 10^7 ml^{-1}. After addition of 100 µg of Zymolyase 20T (from Seikagaku Kogyo Co. Tokyo, Japan) per ml the cell suspension was incubated at 30 ºC [Kitamura and Yamamoto, 1972]. Aliquots were taken after 15 and 30 min of incubation with the enzyme and the spheroplasts were diluted in water to induce hypotonic lyses, later were plated on YEPD. Colonies resistant to the treatment were counted after 7 days of incubation at 28 ºC. For each assay a control reaction omitting the enzyme was performed.

RESULTS

Colony morphology

When wild–type and UVM9 cells were plated on YEPD for 7 days, important differences in morphology and size

were observed. Wild-type strain developed into fuzzy colonies distinguished by dense hyphae formation on the colony surface (Fig. 1A) with abundant pyriform conidia (2.7 x 5 µm) (Fig. 1C). The diameter of the colony was of 7.5 ± 0.1 mm (n = 100). In contrast, UVM9 generated small colonies with scarce fuzzy sectors and a radial growth of 2.8 ± 0.1 mm (n = 100) of diameter (Fig. 1B); clusters of conidia with a diameter of 7 mm instead of typical pyriform conidia were produced (Fig. 1D).

Morphological development of wild-type and UVM9 strains

To investigate whether the mutant is affected in its the ability to develop as hyphae or yeast in response to the pH of the culture medium, the wild-type and UVM9 conidia were grown in YEPD, RV pH 5.5 and 7.2. Aliquots were analyzed microscopically after different times of incubation (12, 18 and 24 h). Consistent with previous reports [Wu *et al*., [Rodriguez del Valle et al ., 1983] when wild-type conidia are transferred to RV pH 5.5 and YEPD they become polarized generating a new hyphal tip. After 12 hrs of incubation in RV pH 5.5, 98% of conidia were germinated while in YEPD medium at 8 hrs, 100% were germinated. A continuous polarized growth resulted in the extension of the hyphae (fig. 2 A and 2B); formation of branches were observed at 24 hrs of incubation in YEPD (2B) while longer incubation time is need to obtain branches in RV pH 5.5 medium. In RV pH 7.2 conidia started the yeast pattern development, bud growth was polarized to the tip (Fig. 3A) leading to tube-like growth followed by budding of the cells. After 12 hrs of incubation 24% of conidia were transformed into yeast and at 18 hrs 84% were observed as budding cells. The yeast form of *S. schenckii* is potentially a multiple-budding cell, though in the conditions that we studied the prevalent form of budding was with one or two buds (Fig. 3A). When the mutant was grown in the same conditions as the wild-type, it developed by polarized extension in RV pH 5.5 and YEPD (2C and D), however was unable to grow as budding yeast-cell in RV pH7.2. Instead, 80% of conidia formed germ tubes in the first 18 hrs followed by an enrichment of hyphae at 24 hrs (Fig. 3B). Longer times of incubation produced a mix population of hyphae and round cells. In the three culture conditions, the steps in development of the mutant were morphologically similar among them. After 24 hrs of growth the wild-type and mutant conidia germinated and produced hyphae. The UVM9 had a limited apical extension, the hyphae were short and thick with period-

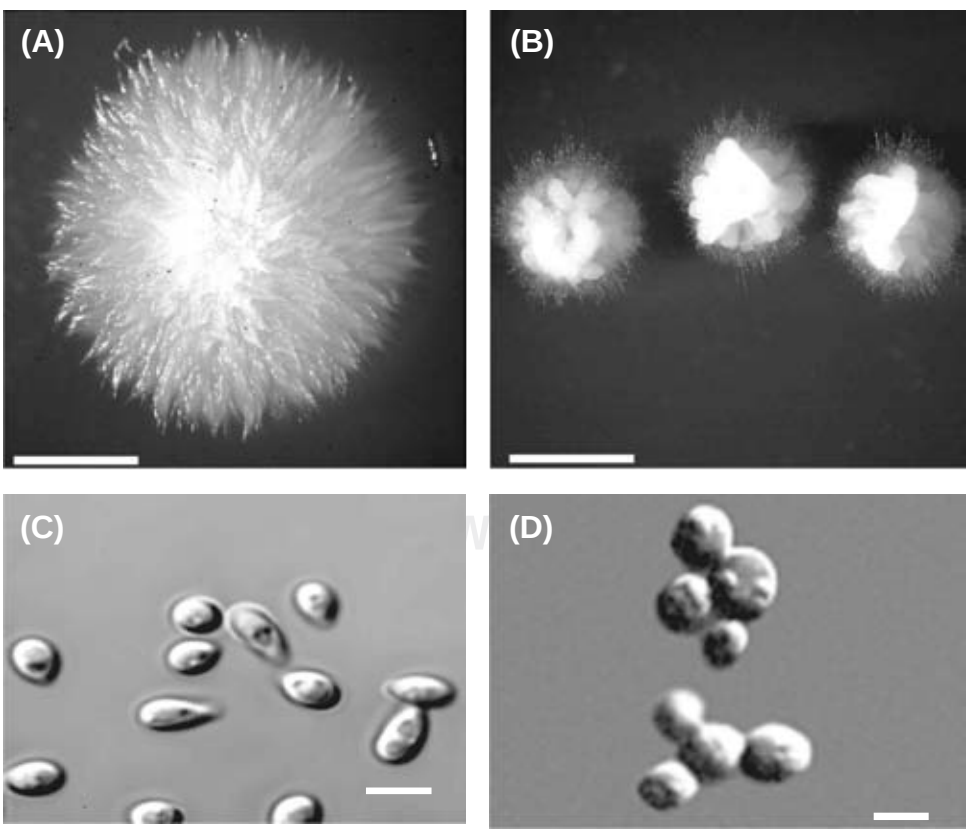

Figure 1. *Colonies had grown 7 days on YEPD at 28 °C.* ***(A)*** *wild-type and* ***(B)*** *UVM9 mutant. The bar corresponds to 2.5 mm. Condia obtained from cultures.* ***(C)*** *wild-type,* ***(D)*** *UVM9 conidia. The bar corresponds to 5 µm.*

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ic slender regions (2C and 2D), whereas in the wild-type generally gave rise to long and straight filaments with branches (Fig. 2B).

From these results we conclude that the UVM9 is able to establish the filamentous morphology however the phenotype suggests a defect in the ability of the UVM9 cells to sustain or conduct a polarized growth. This mutant is also impaired in its capacity to develop yeast-budding cells.

Phenotypic analysis of UVM9 mutant

Cell extension is a regular process in the development of yeast and hyphae [Harris and Momany, 2004], the enzymes required for cell growth are translocated to the cell surface via microfilaments [Yokoyama *et al*., 1990] or microtubules [Steinberg *et al*., 2001]. In order to understand if the impaired growth as budding cell and the reduced hyphae growth is due to alterations in any of the major filament systems, the phenotype of the UVM9 and the wild-type strain were compared by testing sensitivity to drugs that target microfilaments, microtubules and cell wall. Serial dilutions from a suspension of 1 x 10^6 cells ml^{-1} were plated on YEPD containing different drugs concentrations. Colonies were counted after 7 days of incubation at 28 ºC to determine viability.

The primary effect of cytochalasins is the inhibition of actin polimerization [Cooper, 1987]. By growing the wild-type and UVM9 strains on solid media containing various

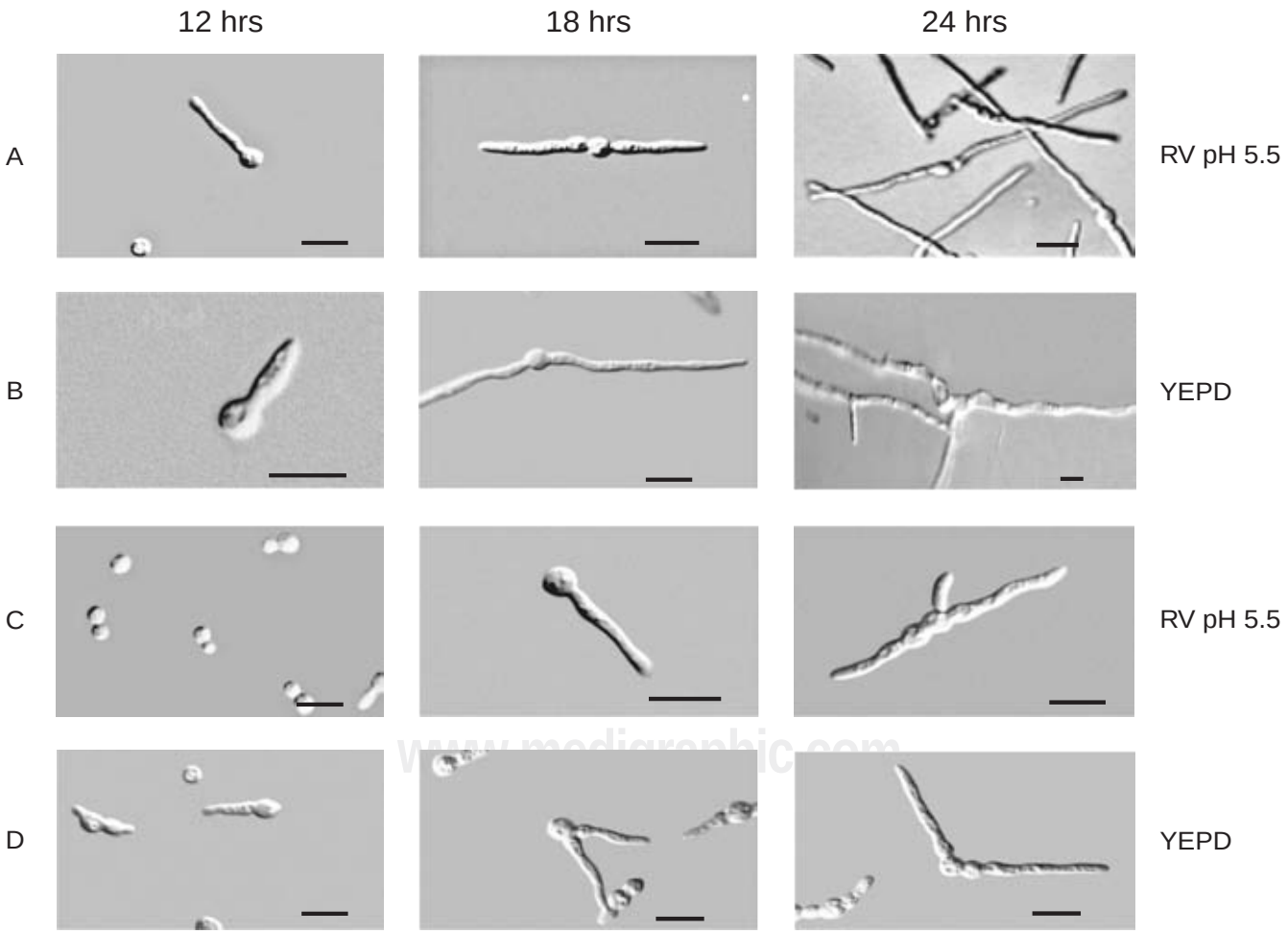

Figure 2. *Induction of mycelium development of* S. schenckii. *Wild type (A, B) and UVM9 (C, D) conidia were culture in RV pH5.5 and in YEPD at 28 °C. Cells were visualized by DIC microscopy.*
Bar = 25 µm.

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concentrations of cytochalasin B (1 to 5 µg ml^{-1}) or by the incubation for 30 min in liquid medium containing 5 µg ml^{-1}, and seeding on YEPD without the drug (not shown). We observed that both strains are resistant to the higher concentration of the drug (Fig. 4A), suggesting that the mutant is not altered in the microfilaments. Benomyl and griseofulvin are drugs that specifically target microtubules (α and β tubulin); these drugs inhibit mitosis in sensitive fungi by disrupting spindle microtubule [Jordan and Wilson, 1998]. Benomyl is a member of the benzimidazole family of microtubule-destabilizing drugs and is known to affect the growth of many tubulin mutants in yeast and inhibits mitosis resembling the actions of colchicines. (Horio and Oakley 2005; Fuchs *et al*., 2005. Griseofulvin inhibits mitosis in fungal cells, altering the rate and extent of micotubules shortening but it has a weak effect in mammalian cells [Panda *et al*., 2005).

Incubation of UVM9 on YEPD plates supplemented with benomyl showed that the mutant was moderately resistant to benomyl (IC_{50} = 0.35 µg ml^{-1}) compared with the wild- type (IC_{50} of 0.17 µg ml^{-1}) (Fig. 4B). In contrast, griseofulvin inhibits severely the growth of the mutant cells (IC_{50} = 339 µg ml^{-1}) while the wild-type strain survived at the higher concentration of 1 mg ml^{-1} (Fig. 4C). These results suggest that the UVM9 strain is affected in microtubule cytoskeleton but probably not in microfilaments.

Cell wall is a dynamic structure and is determinant for cell morphology, defects in the proportion of cell wall components result in alterations in the cell shape. Sensitivity to substances that interfere with the synthesis or assembly of cell wall components can be used as a parameter for changes in cell wall composition in yeast. To test the existence of such changes, in the UVM9 mutant we examine the sensitivity of UVM9 cells to calcofluor white and zymolyase lyses. Calcofluor white is a negatively charged fluorescent dye with antifungal activity; preferentially it binds to chitin on the cell wall and interferes with normal wall assembly preventing co-crystallization of chitin microfibrills with glucan chains [Elorza *et al*., 1983; Surarit *et al*., 1988; Hartland *et al*., 1994; Rowbottom *et al*., 2004].

By growing *S. schenckii* strains on YEPD containing calcofluor we determined that the mutant is resistant to calcofluor at 20 µg ml^{-1}. In contrast the wild-type was sensitive and showed an IC_{50} of 7.5 µg ml^{-1} (Fig. 5A). Resistance to calcofluor suggests a decrease of chitin in the mutant cell wall that may result in the modification of other structural components. In order to distinguish cell wall changes in β-1, 3 glucan of the mutant, we evaluated the zymolyase-induced hypotonic lysis [Herman, 1997]. The zymolyase is a β-1,3-glucanase that digests the cell wall and produce spheroplasts (wall-less cells) that lyses when placed into hypotonic solution [Herman, 1997].

We compared the sensitivity to the lyticase of the unicellular cell shapes of the fungus: conidia and yeast cells from the wild-type strain and the conidia of the mutant.

Aliquots from the cell suspension were plated on YEPD medium after zymolyase treatment and hypotonic lyses. Resistant cells were observed after 7 days of incubation on YEPD at 28 ºC. Conidia from the wild-type and UVM9 subjected to the zymolyase-induced hypotonic lysis, exhibited higher resistance compared with the yeast cells. After 30 min of incubation with the enzyme, 50% of conidia of ei-

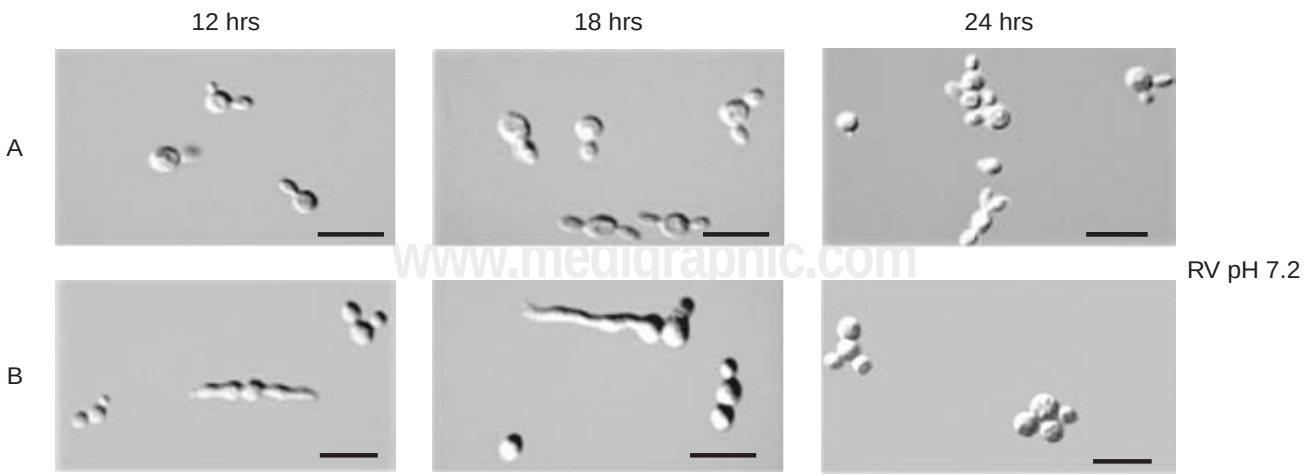

Figure 3. *Induction of yeast development of* S. schenckii. *Wild (A) type and UVM9 (B) conidia were culture in RV pH 7.2 at 28 °C. Aliquots were fixed with 2% of formaldehyde and visualized by DIC microscopy. Bar = 25 µm.*

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ther strain survived to the treatment suggesting that the β-1, 3 glucan structure is not importantly altered in the mutant. Yeast cells were more sensitive to hypotonic lyses after zymolyase incubation, 8.5 min of incubation were sufficient to obtain 50% of survival (Fig. 5B).

DISCUSSION

To initiate the identification of molecules involved in the growth of yeast or hyphae shapes in *S. schenckii*, we report in this work the phenotypic characterization of the

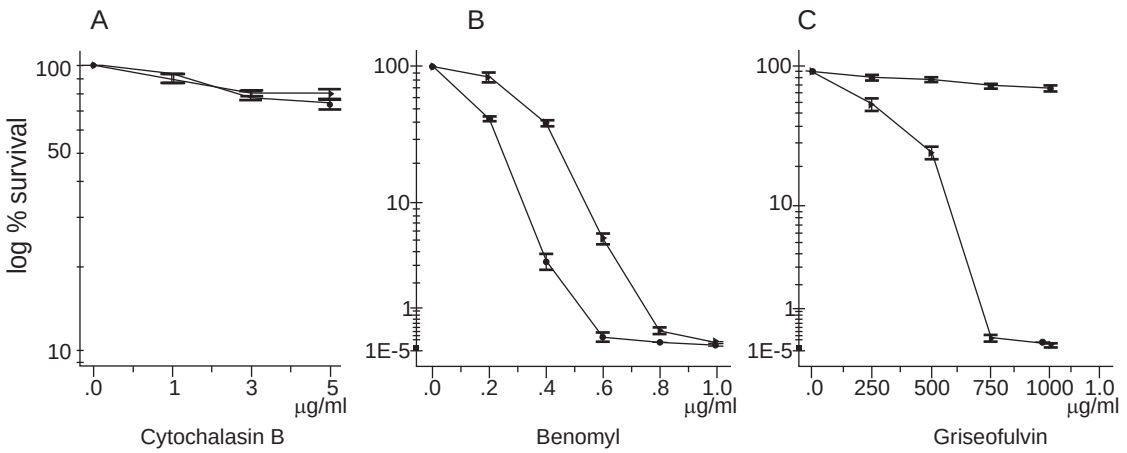

Figure 4. *Survival of* S. schenckii *wild-type and UVM9 conidia in the presence of cytoskeleton disrupting drugs. Cells were plated on YEPD with different concentrations of* ***(A)*** *cytochalasin B,* ***(B)*** *benomyl, and* ***(C)*** *griseofulvin, and incubated for 7 days at 28 °C. Data are means of 5 independent experiments* ● *wild-type,* ▶ *UVM9 mutant.*

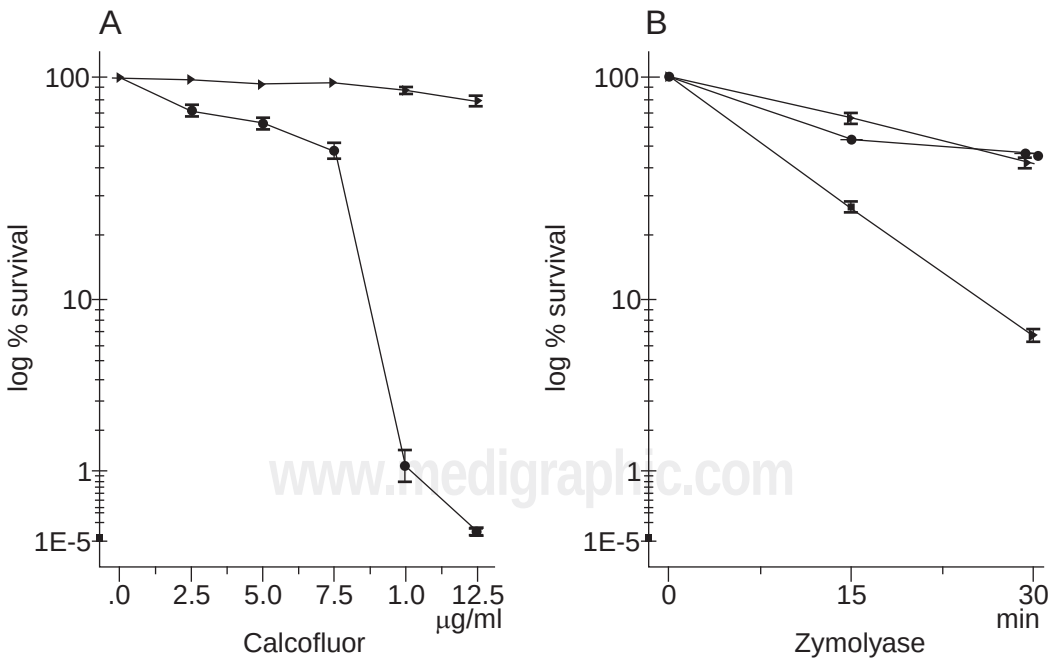

Figure 5. *Survival of* S. schenckii *wild-type and UVM9 mutant conidia in the presence of different concentrations of* ***(A)*** *calcofluor (* ● *wild-type,* ▶ *UVM9 mutant) and* ***(B)*** *after treatment with zymolyase for 15 and 30 min at 30 °C. (* ● *UVM9 yeast-like cells,* ▲ *wild-type yeast-like cells, and* ◆ *wild-type conidia). Data are the mean of 5 independent experiments.*

morphological mutant UVM9, this approach was used because this fungus is not amenable for genetic or molecular manipulation.

By analyzing the dimorphic transition and the resistance phenotypes of UVM9 to compounds that target cytoskeleton and cell wall we can suggest that the altered colony and cell morphology is due to a mutation that hampers the maintenance of polarize growth. The UVM9 mutant developed germ tubes at pH 5.5 and 7.2 however could not maintain the polarized growth giving rise to thick hyphae with limited growth and development of atypical conidia. The failure to properly organize the cytoskeleton of actin or tubulin may result in inefficient transport of vesicles involved in the cells polarized growth, integrity of the cell wall, secretion and endocytosis and during dimorphic transition of pathogenic fungi [Yokoyama *et al*., 1990; Akashi *et al*., *1994*¸ Yokoyama *et al*., 1994; Richards *et al*., 2000; Engqvist-Goldstein, 2003]. The resistance phenotype to drugs that target cytoskeleton and cell wall is a common approach used to study alterations in these structures. The observation that treatment with cytochalasin B [Cooper, 1987] had no effect on the growth and survival of the wild-type and the UVM9 suggest that actin is not altered in the mutant. However to definitively confirm the integrity of actin in the mutant, different actin destabilizing or disrupting drugs as latrunculin A or cytochalasin D should be used. The benomyl resistance and griseofulvin sensitivity phenotype of the mutant suggest that tubulin is responsible of the growth phenotype in the UVM9 hyphae. These drugs bind to tubulin in different sites of the molecule; griseofulvin obstructs microtubule assembly and benomyl binds to the colchicine-binding sites of tubulin arresting cell-cycle. The enhanced sensitivity to griseofulvin and resistance to benomyl supports that tubulin structure is altered. The opposite resistance phenotype observed with these antimicrotubules drugs is the result of the modified drug binding characteristics of the altered tubulin, rather than an increased expression of drug transporters (mdr). Microtubule cytoskeleton provides the structural basis for long distance transport of secretory vesicles [Steinberg and Schliwa, 1993] in the hyphae, any alteration in this structure will impaired the dynamic process of cell wall remodeling during polarized growth of the cell. The main components of the fungal cell wall are mannan, β-1, 6-glucan, β-1, 3-glucan, and chitin. Chitin and β-1,3-glucan are synthesized by different enzyme complexes that are transported to the plasma membrane by a secretory pathway. Changes in cell wall structural integrity of some fungi are expressed as changes in the sensitivity of cells to various inhibitors that stress the wall in different ways [Elorza *et al*., 1983, Surarit *et al*., 1988; Hartland, 1994; Mendoca *et al*., 1976]. Sensitivity to calcofluor has been shown to depend in chitin content of the cell wall of different fungi as described for mutants of *C. albicans* and *S. cereviseae* [Surarit, 1988; DeNobel *et al*., 1990].

Resistance to calcofluor of the UVM9 mutant suggests a decrease in cell wall chitin and probable alteration in the normal wall assembly with glucan chains [Elorza *et al*., 1983; Surarit *et al*., 1988; Hartland *et al*., 1994]. However the similar resistance phenotype to zymolyase suggests that the cell wall do not have substantial changes in its level and structure, which could modify the accessibility of the enzyme to the 1, 3-β-glucan.

In yeast and filamentous shapes, F- actin and microtubules play an important role in the polarized growth. The coordinated interaction of these elements is crucial for many cellular processes including cell locomotion, nuclear migration, cytokinesis, and morphology. The observations presented in this work suggest that a mutation in any of the tubulins (α or β) of the *S. schenckii* UVM9 strain is responsible of the altered morphology of the cell and the impaired polarized growth. Further studies on the cytoskeleton are needed to clarify the genotype of this mutant.

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REFERENCES

1. Kwon-Chung, K.J. &. Bennett, J.E. 1992. Sporotrichosis, pp.707-729. In: K.J. Kwon-Chung. & J.E. Bennett (Eds). Medical Mycology. Lea & Febiger; Philadelphia.
2. Travassos L.R. 1985. *Sporothrix schenckii*, pp. 121-131. In: PJ. Szanizlo (Ed) Fungal Dimorphism with Emphasis on Fungi Pathogenic for Humans. Plenum Press, New York.
3. Alison E. M. Adams & J. R. Pringle 1984. Relationship of Actin and Tubulin Distribution to Bud Growth in Wild-type and Morphogenetic-Mutant *Saccharomyces cerevisiae*. J. of Cell Biol. 934-945.
4. Madhani, H. D. & G.R. Fink. 1998. The control of filamentous differentiation and virulence fungi. Trends Cell Biology. 8:348-353.
5. Xiang X. & M. Plamanny. 2003. Cytoskeleton and motor proteins in filamentous fungi Current Opinion in Microbiology, 6:628-633.
6. Akashi, T., Kanbe, T. & K. Tanaka. 1994. The role of the cytoskeleton in the polarized growth of the germ tube in *Candida albicans.* Microbiology. 140:271-280.
7. Seiler, S., Nargang, F.E., Steinberg, G. & M. Schliwa. 1997. Kinesin is essential for cell morphogenesis and polarized secretion in *Neurospora crassa.* EMBO J. 16:3025-3034.
8. Wu, Q., Sandrock, T.M., Turgeon, B.G., Yoder, O.C., Wirsel, S.G. & J. R. Aist. 1998. A fungal kinesin required for organelle

motility, hyphal growth and morphogenesis. Mol Biol Cell. 9: 89-101.
9. Schuchardt, I., Assmann, D. , Thines, E., Schuberth, C. & G. Steinberg. 2005. Myosin-V, Kinesin-1, and Kinesin-3 cooperate in hyphal growth of the fungus Ustilago maydis. Mol Biol Cell. 11:5191-201.
10. Harris, S.D. & M. Momany. 2004. Polarity in filamentous fungi: moving beyond the yeast paradigm. Fungal Genet Biol. 41:391-400.
11. Resto, S. & N. Rodríguez-del Valle. 1988. Yeast cell cycle of *Sporothrix schenckii*. J Med Vet Mycol. 26: 13-24.
12. Torres-Guerrero, H. & G. Arenas-Lopez. 1998. UV irradiation induced high frequency of colonial variants with altered morphology in *Sporothrix schenckii*. Med Mycol. 36:81-88.
13. Rodríguez-del Valle, N., Rosario, M. & G. Torres-Blasini. 1983. Effects of pH, temperature, aeration and carbon source on the development of the mycelial or yeast forms of *Sporothrix schenckii* from conidia. Mycopathologia. 82:83-88.
14. Kitamura, K. & Y. Yamamoto. 1972. Purification and properties of an enzyme, zymolyase, which lyses viable yeast cells. Arch Biochem Biophys. 53:403-406.
15. Steinberg, G., Wedlich-Soldner, R., Brill, M. & I. Schulz. 2001. Microtubules in the fungal pathogen *Ustilago maydis* are highly dynamic and determine cell polarity. J Cell Sci. 114: 609-622.
16. Cooper, J.A. 1987. Effects of cytochalasin and phalloidin on actin. J Cell Biol. 105:1473-1478.
17. Jordan, M.A. & L. Wilson, 1998. Use of drugs to study role of microtubule assembly dynamics in living cells. Methods Enzymol. 298: 252-276.
18. Horio, T. & B. Oakley. 2005 The role of microtubules in rapid hyphal tip growth of Aspergillus nidulans. Mol Biol Cell. 16:918-926.
19. Fuchs U, Manns I, & G.Steinberg 2005. Microtubules are dispensable for the initial pathogenic development but required for long-distance hyphal growth in the corn smut fungus Ustilago maydis. Mol Biol Cell. 16: 2746-2758.
20. Panda, D., Rathinasamy K., Santra, M. & L. Wilson. 2005. Kinetic suppression of microtubule dynamic instability by griseofulvin: Implications for its possible use in the treatment of cancer. PNAS 102: 9878-9883.
21. Elorza, M.V., Rico, H. & R. Sentandreu. 1983. Calcofluor white alters the assembly of chitin fibrils in *Saccharomyces cerevisíae* and *Candida albicans* cells. J. Gen. Microbiol. 129:1577-1582.
22. Surarit, R., Gopal., P.K & M.G. Shepherd. 1988. Evidence for a glycosidic linkage between chitin and glucan in the cell wall of *Candida albicans.* J. Gen. Microbiol. 134:1723-1730.
23. Hartland, R.P., Vermeulen, C.A., Klis, F.M., Sietsma, J.H, & J.G. Wessels. 1994. The linkage of (1-3)-beta-glucan to chitin during cell wall assembly in *Saccharomyces cerevisiae*. Yeast. 10:1591-1599.
24. Rowbottom, L., Munro, C.A. & N.A. Gow. 2004. *Candida albicans* mutants in the BNI4 gene have reduced cell-wall chitin and alterations in morphogenesis. Microbiology. 150:3243-3252.
25. Herman, PK. & J. Rine. 1997. Yeast spore germination: a requirement for Ras protein activity during re-entry into the cell cycle. EMBO J. 16:6171-6181.
26. Yokoyama, K., Kaji, H., Nishimura, K & M. Miyaji. 1990. The role of microfilaments and microtubules in apical growth and dimorphism of *Candida albicans.* J Gen Microbiol. 136:1067-1075.
27. Akashi, T., Kanbe T., & K. Tanaka. 1994. The role of the cytoskeleton in the polarized growth of the germ tube in Candida albicans. Microbiology. 140:271-280.
28. Yokoyama, K., Kaji, H., Nishimura, K., & Miyaji M. 1994. The role of microfilaments and microtubules during pH-regulated morphological transition in *Candida albicans*. Microbiology. 140:281-287.
29. Richards, K. L., Anders, K. R., Nogales, E., Schwartz, K., Downing, K. & H. Botstein 2000. Structure-function relationships in yeast tubulins. Mol Biol Cell. 11:1887-903.
30. Engqvist-Goldstein, A.E. & D.G. Drubin. 2003. Actin assembly and endocytosis: from yeast to mammals. Annu Rev Cell Dev Biol.. 19:287-332.
31. Steinberg, G. & M. Schliwa. 1993. Organelle movements in the wild type and wall-less fz;sg;os-1 mutants of *Neurospora crassa* are mediated by cytoplasmic microtubules. J Cell Sci. 106: 555-564.
32. Mendonça, L., Gorin, P.A., Lloyd, K.O. & L.R. Travassos. 1976. Polymorphism of *Sporothrix schenckii* surface polysaccharides as a function of morphological differentiation. Biochemistry. 15:2423-2431.
33. DeNobel, J. G., Kils, F. M., Priem, J., Munnik, T & H. van den Ende. 1990. The glucanase-soluble mannoproteins limit cell wall porosity in *Saccharomyces cerevisiae.* Yeast 6:491-499.

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