MYCOLOGY

Scanning electron microscopy characterisation of colonies of Candida albicans morphological mutants

M. PESTI, M. SIPICZKI* and Y. PINTÉR

Department of Genetics and Microbiology, Janus Pannonius University, H-7624 Pécs, POB 266, Hungary and
*Department of Genetics, University of Debrecen H-4032 Debrecen, Hungary

The ultrastructures of colonies of two stable UV-induced morphological mutants and their parental strain of Candida albicans grown on glucose-containing solid medium were investigated by scanning electron microscopy. The structures and ultrastructures of these three types of colonies were determined not only in terms of the proportions of blastospores, hyphae and pseudohyphae, but also with regard to the mode of budding of blastospores and the positions of these particular cell types within the colonies. Hyphae with an atypical appearance and branching characters were observed both in regular-wrinkled and in irregular-wrinkled mutant colonies. Smooth colonies of the parental strain and the mutants exhibited the same hyphal network within the agar, suggesting that micro-environmental factors in the agar overcame the effects of these mutations.

Introduction

Candida albicans is a diploid, naturally heterozygous, opportunistic pathogen. It is an imperfect yeast capable of a yeast-to-hyphal transition (dimorphic transition) induced by environmental factors and a variety of high-frequency heritable, reversible, strain-dependent, phenotypic transitions (frequently called switching), resulting in differences in colony morphology [1-3].

The induction and characterisation of Candida morphological mutants have shed light on some interesting aspects of these two properties. Certain somatic hybrids formed by protoplast fusion of benomyl-induced morphological mutants resulted in two complementation groups representing two genes relevant for dimorphism, whose alteration interfered with the correct transition from blastospore to mycelium [4]. Karyotype analysis of UV-induced C. tropicalis morphological mutants suggested the occurrence of chromosomal re-arrangements associated with morphological mutation [5]. The first report of a nitrosoguanidine-induced C. albicans mutant that was stable at ambient temperatures seems to afford a promising possibility for identification of the genes responsible for the yeast-mycelial transition [6].

The aim of the present study was to apply scanning electron microscopy (SEM) to investigate the ultrastructure of individual colonies of two stable UV-induced C. albicans morphological mutants and their parental wild-type strain and to compare these observations with earlier findings relating to the ultrastructure of colonies of different origins [7-10].

Materials and methods

Strains, culture conditions and isolation of mutants

The wild-type strain of C. albicans (ATCC 64385) was used to obtain morphological mutants as described previously [11]. Identification of this strain and the mutants obtained was achieved by germ-tube formation in bovine serum and by determination of the fermentation and assimilation spectra.

The medium used (YPG) contained (g/L): yeast extract 3, peptone (Difco) 5, glucose 10 and Bacto Agar (Difco) 20, at pH 6.5.

After UV-mutagen treatment, one star-shaped mutant colony (Fig. 1b) was multiplied by applying a single cell descendant method. Two stable segregants, one regular-wrinkled (Fig. 1c) and one irregular-wrinkled (Fig. 1d), were selected after culturing 10 times and compared with the smooth wild-type strain (Fig. 1a).

Cells were examined and photographed with an
Colonies of C. albicans strain ATCC 64385 and mutants. The basic original smooth phenotype (a) was irradiated with UV light, causing 92% cell death. One of the unstable morphological mutants with a star phenotype (b) was segregated, resulting in stable regular-wrinkled (c) and irregular-wrinkled segregants (d). Nuclei of cells visualised with DAPI originated from the irregular-wrinkled colony (e). Olympus BH-2 microscope. Nuclei were visualised with DAPI (4,6-diaminido-2-phenylindole), as described elsewhere [12].

One-week-old single colonies of these three lines, grown on YPG medium at 30°C, were used to determine the cell types present in the colonies by light microscopy and to prepare samples for SEM at the same time.

Preparation for SEM

Samples were prepared according to the method of Radford et al. [7]; an IFC-1100 Ion Sputter (JEOL) was used to coat samples with gold and they were viewed by SEM (JEOL 6300).

Results

Colony types

The colony types investigated in this study are depicted in Fig. 1.

Smooth colonies of wild-type strain

Over the agar surface, the whole colony consisted of blastospores only. However, three layers could be seen in a cross-fractured section of the colony (Fig. 2a). On the surface of the colony, resting, multipolar budding blastospores which were homogeneous in diameter were visible (Fig. 2b). Under the surface, there was a 100-µm spongy layer, where unipolar budding (two or three cells in a row) alternating with the multipolar budding of cells created small cavities (Fig. 2c). Over the agar surface, there was a 200-µm dense layer, where multipolar budding predominated. The channel in the middle of the picture was an artifact resulting from the preparation procedure (Fig. 2a). The whole colony had a branching network of capillaries from the top to the bottom. Under the agar surface, there was a fine network of true hyphae, with few blastospores until a depth of 400 µm (Fig. 2d).

Regular-wrinkled colonies

These colonies contained 6% hyphae and pseudohyphae and 94% blastospores. The surface was rippled (Fig. 3a). The surface of the colonies consisted only of blastospores which were heterogeneous in diameter. Active growth and multipolar budding were observed (Fig. 3b). Under the surface, there was a 100-µm thick homogeneous layer composed of more or less elongated blastospores, whose alternating unipolar (two to five cells in a row) and multipolar budding produced a spongy structure (Fig. 3c). At the centre of the colonies, hyphae and pseudohyphae-like structures appeared. Some were thin (0.25–0.5 µm) and relatively long (6–20 µm) atypical branching hyphae growing out from blastospores, continuing in blastospores or hyphae cells at the other end.
ULTRA STRUCTURE OF COLONIES OF CANDIDA ALBICANS BY SEM

Fig. 2. SEM of smooth colonies of C. albicans: (a) cross-fractured face of the colony (×230); blastospores on the surface of the colony (×750); (b) the sub-surface layer of the colony showing unipolar and multipolar budding of blastospores (×400); (c) hyphal network and some blastospores growing into the agar (×230).

suggesting that they were viable (Fig. 3d). All contained nuclei (Fig. 1e), some of which were elongated.

Irregular-wrinkled colonies

These contained 45% hyphae, 5% pseudohyphae and 50% blastospores. Throughout the colonies, hyphae and pseudohyphae were the predominant cell type. Towards the centres of the colonies, the diameter of the cavities increased (Fig. 4a–d). The surface was covered with hyphae, some pseudohyphae and a few budding blastospores (Fig. 4b). Aerial hyphae frequently appeared to be connected to two parts of the surface. They were cable-like structures consisting of hyphae...
Fig. 3. SEM of the regular-wrinkled mutant colonies of *C. albicans*: (a) cross-fractured face of the colony (×150); (b) blastospores heterogeneous in diameter on the surface of the colony (×750); (c) the sub-surface layer of the colony showing unipolar budding (×2700); (d) atypical hyphae in the middle of the colony (×3500).

with some blastospores on their surface (Fig. 4c). Under the surface of the colony, there was a denser layer with a cavity system. This was built up with branching hyphae and some budding blastospores (Fig. 4d). In the middle of the colonies, long (10–60 μm), branching atypical hyphae of variable diameter (0.3–0.7–1.2–3.8 μm) could be seen (Fig. 4e). They were also observed in the regular-wrinkled colonies (Fig. 3d). In this case, they appeared more frequently and their branching was more pronounced.

The regular- and irregular-wrinkled colonies exhibited almost the same hyphal system in the agar as that observed for the smooth colonies.
Fig. 4. SEM of the irregular-wrinkled mutant colonies of *C. albicans*: (a) cross-fractured face of the colony (×130); (b) budding blastospores and hyphae on the surface of the colony (×2000); (c) aerial hyphae connecting two parts of the surface (×1200); (d) sub-surface layer showing hyphae and some budding blastospores (×1100); (e) heavy network of atypical hyphae in the middle of the colony (×1600).

Discussion

This study investigated the colony ultrastructures of two mutagen-induced, stable morphological mutants of *C. albicans* and their parent strain. In agreement with earlier findings [7, 9], the smooth colonies consisted only of blastospores and budding was observed only at the bottom of the colonies.

No adhesive extracellular material [9], intercellular matrix [10] or communicating filaments [7] were observed. The speculation by Odds [1] that these types
of fibrillar materials might originate from outer cell-wall coat appears to be correct. It has been proved experimentally that the appearance of a fibrillar matrix depends on the applied carbon source [8] and can be induced by nystatin treatment [11]. Following polyene treatment, the observed matrix contained intracellular materials resulting from plasma membrane disintegration. The carbon source influenced the mode of budding, bud scar morphology, surface topography [8] and colony morphology [5].

The budding of blastospores was multipolar at the surface of smooth colonies and alternated in a unipolar and multipolar way under the surface, creating a new type of ultrastructural layer not only in the smooth colonies, but also in the regular- and irregular-wrinkled colonies, which might have been caused by micro-environmental factors (e.g., the supply of nutrients and oxygen). This observation contrasts with previous reports [7–10].

The readily observable microcapillary and cavity system of the three types of colonies could play a role in nutrient transport in vitro. At the same time, the coherent network of hyphae and the central pores of their intercellular septa which might provide intercellular communication [6] in the irregular-wrinkled colonies, might explain the high number of budding blastospores on the surfaces of these colonies.

Surface wrinkling was explained earlier by the development of the surface hyphal cells [7]. In the present study, hyphae were absent at the surface of the regular-wrinkled colonies, but appeared in the middle of colonies, resulting in the same phenomenon, which was influenced by the presence of a unipolar budding layer under the surface. The three colony types examined had developed the same hyphal network in the agar, in contrast with earlier findings, where no colonisation in the agar [3] or only an irregular chain of blastospores [7] was observed. One possible explanation is that the mutations that resulted in various colony structures were overcome by micro-environmental factors.

This study has demonstrated that the different colony types and their ultrastructures cannot be explained only in terms of the three main morphological forms of cells described in switching colonies [7]. The type of budding of the blastospores, the positions of hyphae within colonies and micro-environmental factors affecting the colonies in the agar also influence the colony morphology in vitro.

The observed phenomena relating to the colony types and their ultrastructures might have been the results of a multifactorial system. The vector of these factors may be determined or modified by dimorphic transition, phenotypic switching or inherited alterations of genetic material. However, it should be emphasised that the switching of the parental C. albicans strain and the chromosomal re-arrangement described earlier [5] were not observed under the conditions applied to explain alterations in colony morphology detected in these mutants by electrophoretic karyotype analysis [12]. The described phenomenon depended on environmental factors (e.g., unipolar and multipolar budding of blastospores) and mutations. These in-vitro results may help to elucidate the pathogenicity processes and different types of colonisation of C. albicans in various host organs in vivo.

Examination of the changes in isozyme and random amplified polymorphic DNA patterns and in electrophoretic karyotypes of these mutants of C. albicans is under way [13].

We are grateful to F. Kaposvári of the Electronmicroscopy Laboratory, Medical University, Pécs, Hungary, for technical assistance.

References