SALT INDUCED CHANGES IN CELL WALL PROTEIN POPULATION OF *Hortaea werneckii* and *Wallemia ichthyophaga* - PRELIMINARY RESULTS

Janja Zajc, Tomaž Vaupotič, Ana Plemenitaš, Nina Gunde-Cimerman

"EMERGING POTENTIAL OF BLACK YEASTS"

3rd Meeting of ISHAM Working groups on Black Yeasts and Chromoblastomycosis

Slovenia, Ljubljana, May 16

CONTENTS

1. Two eukaryotic models of halophily studies
2. Life in hypersaline environments
3. Cell wall structure
4. Aim, methods
5. Preliminary results
6. Future perspectives

FUNGI IN HYPERSALINE ENVIRONMENTS

Evaporation ponds in Sečovlje solar salterns.

- fungi are active inhabitants of hypersaline environments

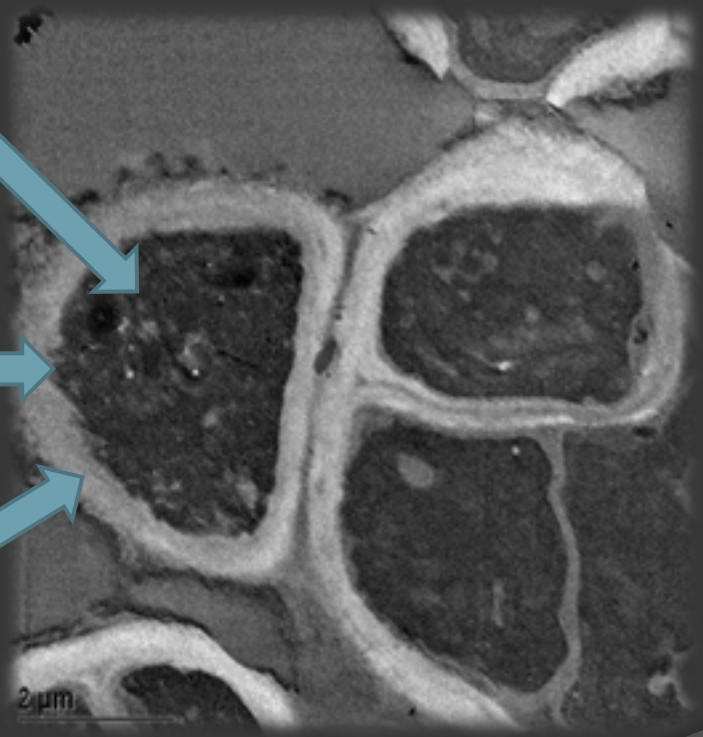
Hortaea werneckii	***Wallemia ichthyophaga***
Ascomycetous black yeast	Basidiomycetous fungus
Extremely halotolerant	Halophilic
Current model for halophily studies	Future model for halophily studies?

LIFE IN HYPERSALINE ENVIRONMENTS REQUIRES ADAPTATIONS OF THE CELL

Osmolyte synthesis and/or accumulation

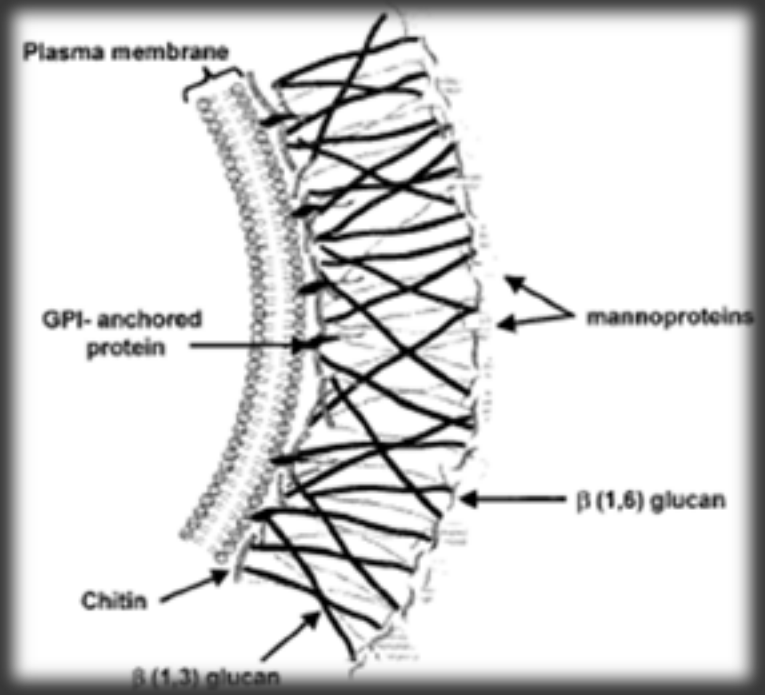
Cell membrane adjustments

Cell wall structure adjustments!



Cell wall is the first line of defence against environmental stress!

FUNGAL CELL WALL STRUCTURE



Schematic of fungal cell wall.

(Selitrennikoff, C. P. (2001). "Antifungal proteins." AEM **67**(7): 2883-2894)

CW is bilayered structure:

Skeletal layer:

- polysaccharides:
  - β(1,3) glucan
  - β (1,6) glucan
  - chitin

Mechanical strength

Fibrillar layer:

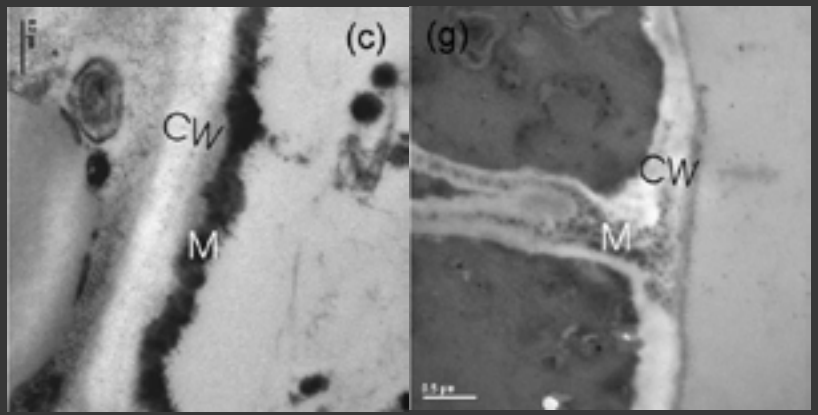
- **proteins (CWPs)**
  - mostly glycoproteins

Involved in mating, biofilm formation, protection against stress.

CWPs are in direct contact with the environment!

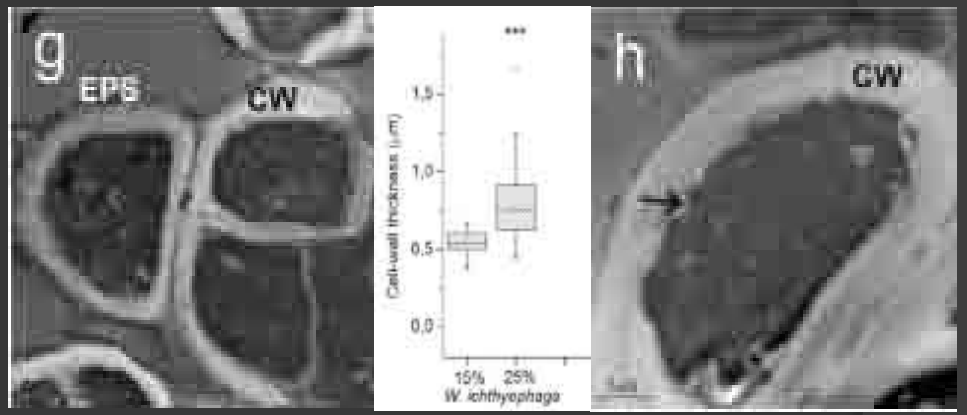
CELL WALL OF *H. werneckii* AND *W. ichthyophaga*

- Melanized
- Non-melanized
- Thick



Detail of *H. werneckii* cells (5% (c) and with 20 % (g) NaCl in liquid media).

(Kogej, T., M. Stein, et al. (2007). "Osmotic adaptation of the halophilic fungus Hortaea werneckii: role of osmolytes and melanization." Microbiology **153** (Pt 12): 4261-73)



***W. ichthyophaga* cells (in liquid media with 15% (g) and 25% (h) NaCl).**

(Kralj Kuncic, M., T. Kogej, et al. (2010). "Morphological response of the halophilic fungal genus Wallemia to high salinity." AEM **76**(1): 329-37)

Nothing is known about cell wall proteins!

AIM OF THE STUDY

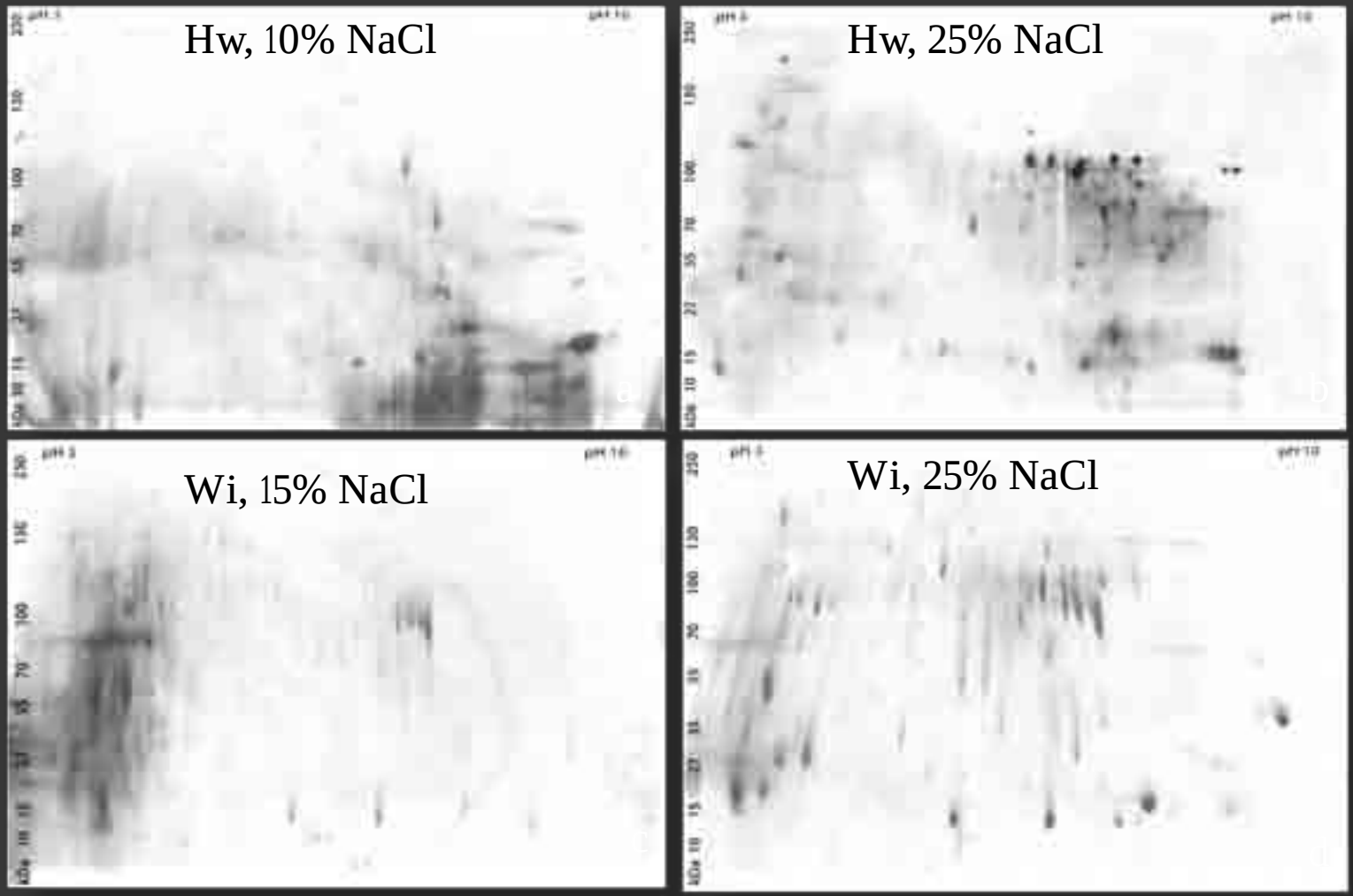
- Identification of **salt induced CWP**s at high NaCl concentration in
  - *H. werneckii*
  - *W. ichthyophaga*

METHODS

- CW isolation and CWPs extraction at moderate and high NaCl concentration
- Separation of the CWPs with 2D SDS-PAGE
- Identification of the CWPs with LC/MS/MS

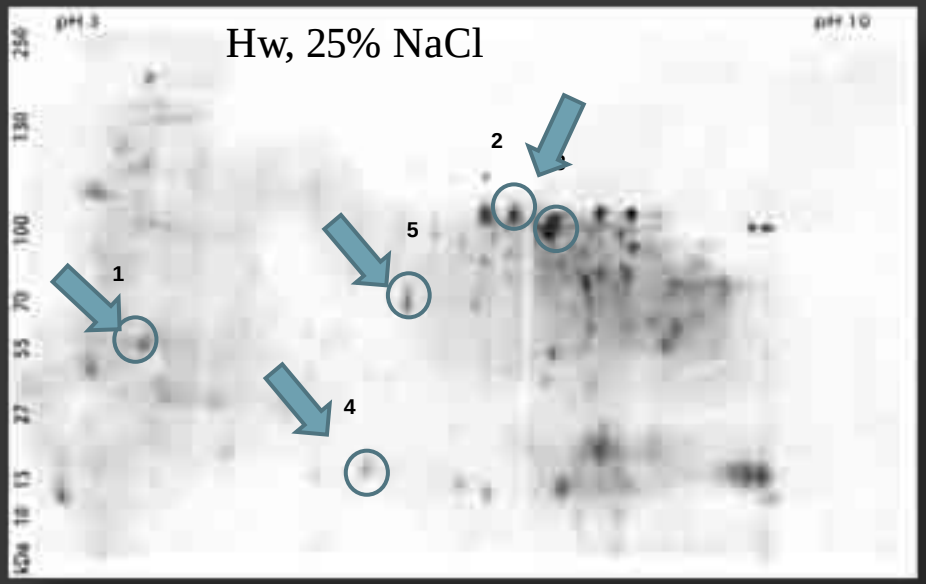
PATTERNS OF CWPS AT MODERATE AND HIGH SALINITY ARE DIFFERENT

Moderate salinity **High salinity**



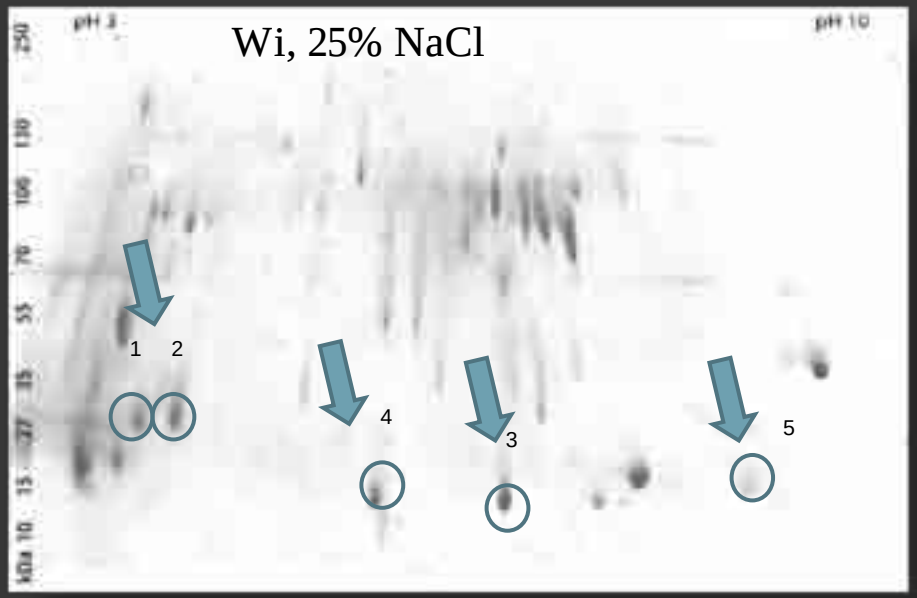
2DEF images of *H. werneckii* and *W. ichthyophaga* CWPs

H. werneckii: CWPs DIFFERENTIALLY EXPRESSED AT 25% NaCl



Spot	Protein identification	GenBank best hit
1	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	*S. cerevisiae*
2	hypothetical heat shock protein 70Kda	*Neurospora crassa*
3	Heat shock protein (HSP70)	*Neurospora crassa*
4	NADP-dependent mannitol dehydrogenase	*Cladosporium fulvum*
5	ENO_ALTAL Enolase	*Alternaria alternata*

W. ichthyophaga: CWPs DIFFERENTIALLY EXPRESSED AT 25% NaCl



Spot	Protein identification	GenBank best hit
1	Glyceraldehyde-3-phosphate dehydrogenase	*S. cerevisiae*
2	Glyceraldehyde-3-phosphate dehydrogenase	*S. cerevisiae*
3	Glyceraldehyde-3-phosphate dehydrogenase	*S. cerevisiae*
4	Glycosyl transferase	*Symbiobacterium thermophilum*
5	Protein involved in cell morphogenesis and proliferation	*S. cerevisiae*

CONCLUSIONS

- Higher number of CWPs expressed at high salinity
- GAPDH, HSP and two allergens upregulated at higher salinity in *H. werneckii.*

Are these proteins truly part of cell wall proteome?

ARE THESE PROTEINS TRULY PART OF CELL WALL PROTEOME?

"Traditional cytoplasmic proteins, which were once considered to be contaminants in fungal cell wall preparations are now gaining acceptance as true cell wall constituents."

Bowman S.M. And Free S. J. 2006. The structure and synthesis of the fungal cell wall. BioEssays 28:799-808

Identification of a Glucan-Associated Enolase as a Main Cell Wall Protein of *Candida albicans* and an Indirect Target of Lipopeptide Antimycotics

Letizia Angiolella, Monica Facchin, Annarita Stringaro, Bruno Maras, Nicola Simonetti, and Antonio Cassone

JOURNAL OF BACTERIOLOGY, Aug. 1997, p. 4992–4999
0021-9193/97/$04.00+0

Vol. 179, No. 16

The Glycolytic Enzyme Glyceraldehyde-3-Phosphate Dehydrogenase *of Candida albicans* Is a Surface Antigen

JOURNAL OF BACTERIOLOGY, Aug. 1996, p. 4724–4726
0021-9193/96/$04.00+0

Vol. 178, No. 15

Members of the Hsp70 Family of Proteins in the Cell Wall of *Saccharomyces cerevisiae*

JOSÉ LUIS LÓPEZ-RIBOT AND W. LAJEAN CHAFFIN*

Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, Texas 79430

Received 21 March 1996/Accepted 16 May 1996

Western blot (immunoblot) analysis of cell wall and cytosolic extracts obtained from parental and *ssa1* and *ssa2* single- and double-mutant strains of *Saccharomyces cerevisiae* showed that the heat shock protein 70 (Hsp70) products of these genes, previously thought to be restricted to the cell interior, are also present in the cell wall. A cell wall location was further confirmed by indirect immunofluorescence with intact cells and biotinylation of extracellular Hsp70. Hsp70s have been implicated in translocation across the membrane and as molecular chaperones, and changes in the profile of cell wall proteins suggested that these proteins may have a similar role in the cell wall.

FUTURE PERSPECTIVES

- Avoidance of intracellular protein contamination with improved methods of CWPs extraction
  - **Cell shaving method**: CWPs of intact cells are proteolytically digested and analized by LC/MS/MS
- Comparison of CWPs at moderate and high salinity.

TO BE CONTINUED...

THANK YOU FOR YOUR ATTENTION.

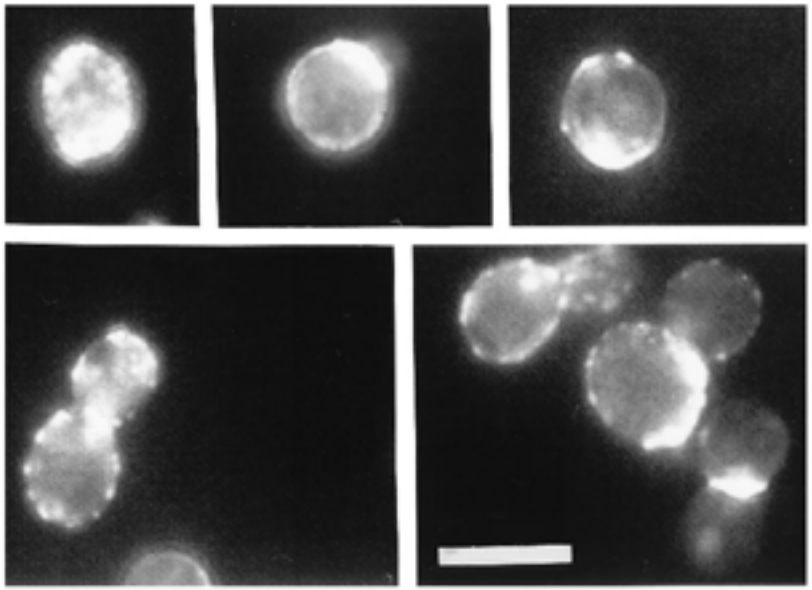


FIG. 3. Surface localization of Hsp70. PAb 343 was used in an indirect immunofluorescence assay with cells of the parental strain, T211. All cells were fluorescent, and images were obtained with a cooled charge-coupled device camera. The digital image was converted to a tagged image file format, processed for brightness and contrast, scaled (imgworks; Silicon Graphics Inc., Mountain View, Calif.), and printed (Codonics printer; Codonics Inc., Middleburg, Ohio). Bar, 5 μm.

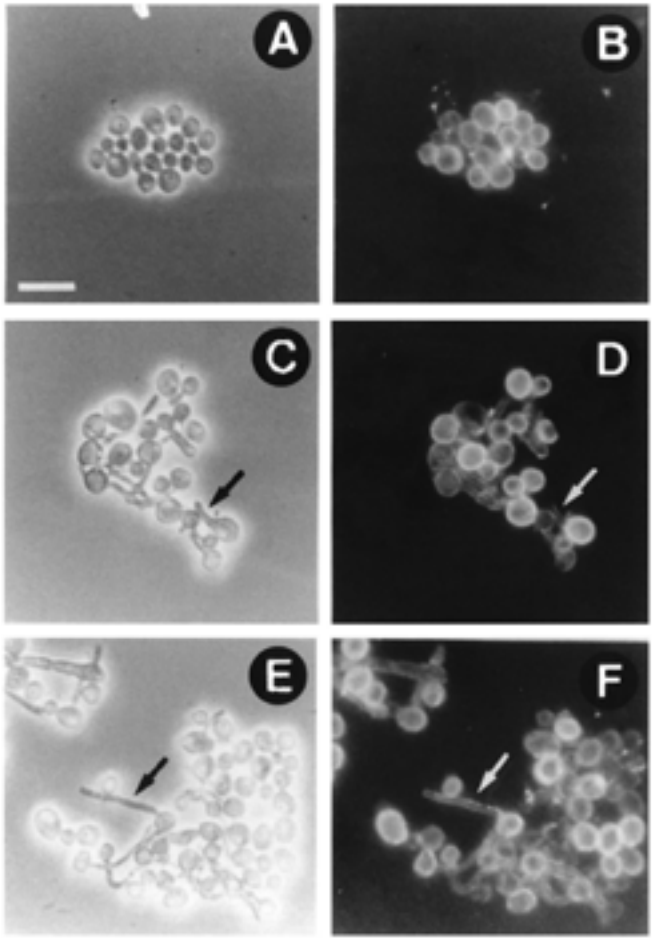


FIG. 3. Surface localization of GAPDH by IIF with PAb anti-CA-GAPDH. Blastoconidia (A and B) and germinated blastoconidia (C to F) were incubated with the PAb anti-CA-GAPDH and FITC-conjugated goat anti-rabbit Ig as described in Materials and Methods. (A, C, and E) Phase-contrast microscopy; (B, D, and F) UV illumination (fluorescence). Note the fluorescence at the blastoconidial surface (B). The germ tube surface visualized by phase-contrast microscopy (C and E [arrows]) exhibited a weak fluorescence (D and F [arrows]). Bar, 10μm.