Viability and Versatility of the Yeast Cell

Michelle J. Henry-Stanley and Carol L. Wells
University of Minnesota, Minneapolis
wells002@umn.edu

Yeasts are single-celled eukaryotic microorganisms (generally about 5 to 10 microns in diameter) that divide by a budding process and are classified with the fungi. Yeast cells are ubiquitous in our environment and can be found on plants and in soil and water. Yeasts have considerable importance in industrial and agricultural settings. *Saccharomyces cerevisiae* (Figure 1) is also known as "bakers yeast" or "brewers yeast." Specific strains of yeast are used to make pastries, bread, beer, ale, wine, distilled spirits, and industrial alcohol. In the paper industry, *Candida utilis* is used to break down the sugars from processed wood pulp. Yeast cells are also nutritious. In some societies, "cloudy" beer (containing yeast cells) provides essential B vitamins, minerals, and amino acids. For over a century, yeasts have been fed to farm animals in a variety of formulations that include fermented mash as well as by-products from breweries and distilleries, and specific yeast products are commercially produced for animal feed. In these commercial endeavors, assessing yeast viability can be critically important.

The ubiquitous yeast can also be found as resident members of the microbial flora of man and animals. The normal human microflora includes several genera of yeast, but the genus *Candida* is likely most important. *Candida* species can be isolated from the skin, oral cavity, and the genitourinary, alimentary, and respiratory tracts of most individuals. Although they are typically considered harmless commensals, yeasts are also important in human disease. Some species are called "opportunistic pathogens" because they can take advantage of specific opportunities (*i.e.*, clinical conditions) to cause disease. These clinical conditions include immunosuppression, as well as antibiotic-induced elimination of competing bacterial flora. The elimination of microbial competition facilitates overgrowth of *Candida* species normally present in low numbers.

It should be emphasized that even though *Candida* species are found in the normal microbial flora of essentially all individuals, some have proposed that asymptomatic colonization with *Candida* is associated with a disease termed either Candida Hypersensitivity Syndrome, or Candida-Related Complex, or Polysystemic Candidiasis, or Chronic Candidiasis. This syndrome is allegedly associated with broad-spectrum antibiotic therapy, oral contraceptives, diets rich in yeast, and pregnancy. Although there are numerous uncritical publications on this topic, no peer-reviewed data in major journals confirm the existence of this syndrome, even though brief communications (that lack peer- reviewed data) have appeared in major journals. Recommended therapies for this syndrome include long-term administration of antifungal drugs, elimination of yeast from the diet, and *Candida* allergy shots. There are no data published in peer-reviewed journals verifying that these therapies are effective. A clear and factual explanation of this topic can be obtained at the website in reference 4. This website is dedicated to providing a wide range of scholarly peer-reviewed contemporary and historical information regarding fungi. The general goal of the website is to promote an understanding of fungi and the ways fungal diseases of humans, animals, and plants affect people throughout the world. The editorial board includes some of the most highly respected mycologists in the scientific community.

In clinical medicine, *Candida* species can cause a spectrum of diseases ranging from superficial infections (such as skin infections, oral infections, and vaginitis) to systemic life threatening systemic infections that involve the blood stream, liver, spleen, kidneys, *etc.* The *Candida* species most frequently associated with human disease include *C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*. *Candida* species are currently the fourth most common cause of nosocomial (hospital acquired) blood stream infection. These systemic infections are life threatening. Despite appropriate antifungal therapy, associated mortality is high and ranges from 38% to 75%. Patients at highest risk for systemic candidiasis include neonates, diabetics, and AIDS patients, but most cases occur in immunosuppressed patients, trauma patients, and postsurgical patients. A large proportion of cases occur in intensive care units. Risk factors include decreased numbers of white blood cells, prolonged physiologic support, vascular catheters, broad spectrum antibiotics, parenteral nutrition, hemodialysis, mucosal colonization, abdominal surgery, damage to the gastrointestinal tract, burns, and corticosteroids. Other risk factors associated with mortality include age, renal failure, hepatic failure, postoperative shock, and respiratory disease syndrome. Due to the high mortality associated with systemic disease caused by *Candida* species, coupled with the ineffectiveness of available antifungal therapy, many researchers are studying the mechanisms by which this typically harmless commensal fungus causes systemic disease. Here, the long-term goal is to identify therapies to interfere with disease initiation in high risk patients.

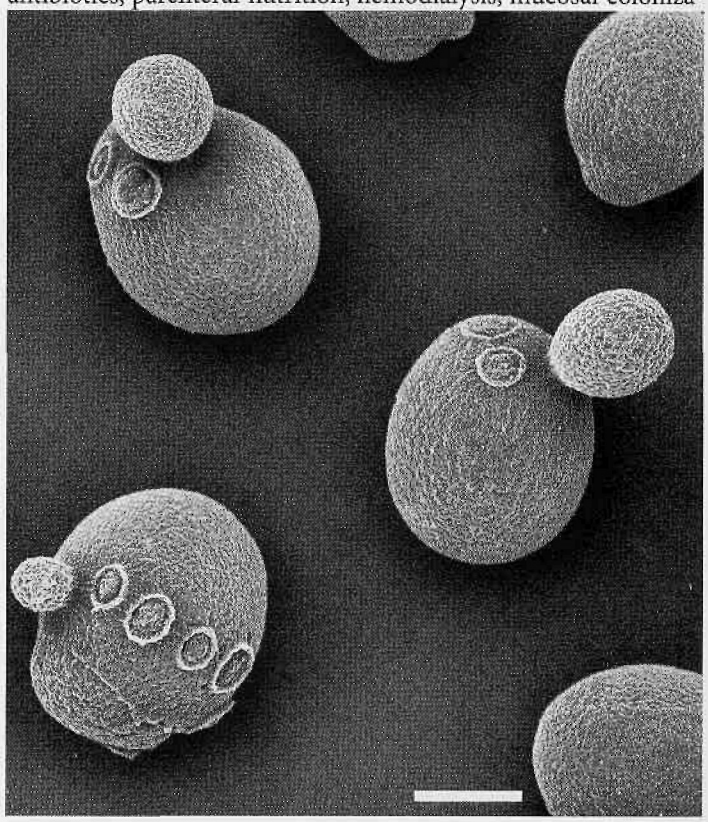


Figure 1. *Field emission scanning electron micrograph of Saccharomyces cerevisiae showing budding yeast cells. Mother yeast cells have one or more prominent "bud scars" marking sites where a budding daughter cell was released from the mother cell. Scale bar = 1.5 microns.*

Thus, in a variety of settings (industrial, agricultural, biomedical), the ability to assess yeast viability is of great importance.

6 MEGAPIXELS

High Definition Digital TEM Cameras

AMT's XR60 & XR100

TEM INTEGRATION

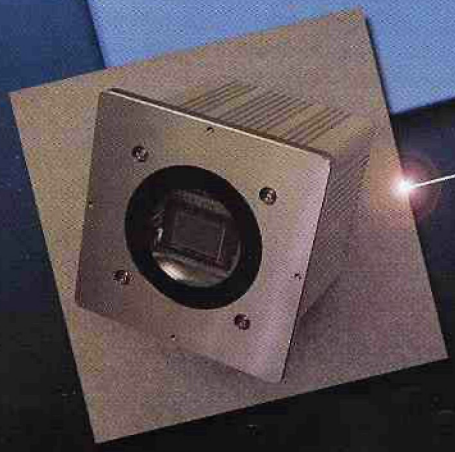
FASTEST DISPLAY

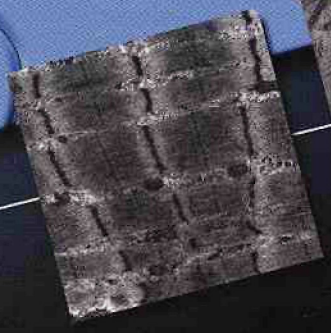
LARGEST FIELD OF VIEW

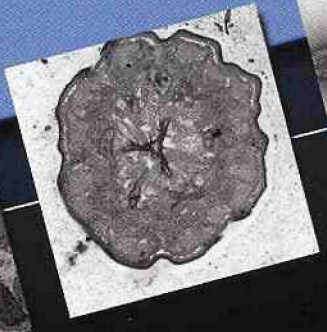
BUILT-IN RELIABILITY

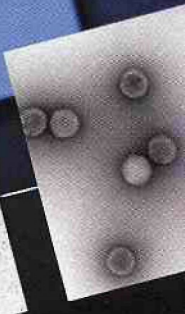
PROVEN SUPPORT

SIDE MOUNT & BOTTOM MOUNT









AMT

Advanced Microscopy Techniques Corporation
3 Electronics Avenue • Danvers, MA 01923
978-774-5550 • www.amtimaging.com

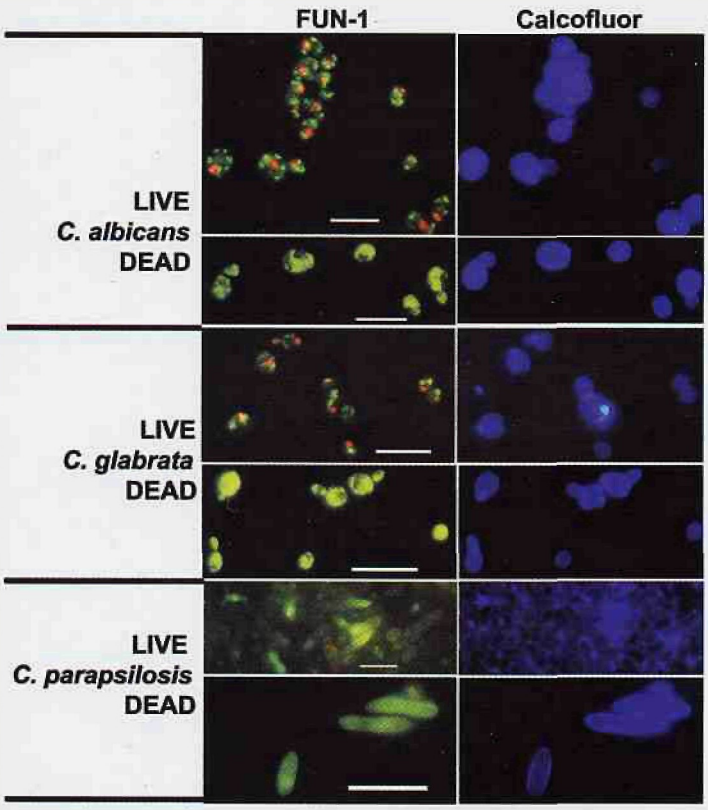


Figure 2. *Three species of live and dead Candida (C. albicans, C. glabrata, and C. parapsilosis) following staining with the FUN-1 LIVE/DEAD® Yeast Viability Kit. The cell walls of both live and dead yeast appear bright blue under a DAPI filter following staining with calcofluor. Under a fluorescein filter, viable yeast cells contain prominent orange-red CIVS vacuoles (indicating metabolic activity) while dead yeast cells have diffuse yellow-green staining. C. albicans yeast cells are about the size of a human white blood cell. Note the relative small size and prominent bud scars (which stain intensely with calcofluor) of C. glabrata. C. parapsilosis cultures contain elongated cells, and live cells tend to clump and have a relatively low level of metabolic activity. Scale bars = 10 microns.*

Classical microbiological techniques of colony counting are cumbersome and time-consuming and may not accurately detect slow-growing or non-dividing yeast. Molecular Probes, Inc. (Eugene, OR) has developed a family of fluorogenic probes that produce differential staining patterns in live and dead yeast cells. This family of fluorochromes is exemplified by the FUN-1 [2-chloro-4-(2,3-dihydro-3-methyl-(benzo-1,3-thiazol-2-yl)-methylidene)-1-phenylquinolinium iodide] stain, which appears to work well with all genera and species of yeast tested to date. Molecular Probes LIVE/DEAD® Yeast Viability Kit combines the FUN-1 stain with Calcofluor White, an ultra-violet excitable dye that has long been used by mycologists as a marker of fungal cell walls. Using this kit, scientists should be able to easily distinguish, often in less than 30 minutes, live and dead fungal cells in a wide variety of industrial, agricultural, and biomedical samples.

Free FUN-1 is essentially nonfluorescent in aqueous solution. FUN-1 stains nucleic acids, producing diffuse green to green-yellow fluorescence in membrane-compromised dead yeast cells. In metabolically active yeast, Cylindrical IntraVacuolar Structures (CIVS) are produced after less than one-hour exposure to FUN-1. These structures often appear to move within a vacuolar space and are orange-red in color. The transport of FUN-1 stain from the cytosol to the vacuole does not occur in nonviable yeast cells, and CIVS are therefore not seen in dead yeast cells. CIVS are approximately 0.5 to 0.7 microns in diameter and from one to several microns in length. Formation of these structures requires both the plasma membrane integrity and metabolic capacity of viable yeast. According to the product insert, a fluorescein filter set with an excitation of about 480 nm and emission ≥530 nm should be suitable to view the FUN-1 stain. Fungal cells stained with Calcofluor White can be viewed with filters appropriate for DAPI and fungal cell walls appear bright blue. Simultaneous viewing of FUN-1 and Calcofluor White can be achieved with a multipass filter set. Using the two dyes together provides a clear visualization of the total population of yeast cells stained with Calcofluor White, and distinguishes between dead yeast cells with a diffuse yellow-green fluorescence and metabolically active yeast cells with cylindrical red-fluorescent structures in cytoplasmic vacuoles.

Figure 2 presents three species of *Candida* cultivated at 30°C overnight at 180 rpm in a minimal broth medium supplemented with 2% glucose. The yeast cells were washed once and a portion was heat-killed at 80°C for 1 hour, with lack of viability confirmed by lack of growth on a suitable agar medium, namely Sabouraud dextrose agar. Both live and dead yeast were then stained with the FUN-1 LIVE/DEAD® Yeast Viability Kit, using a concentration of 1 x 10^7 yeast cells in a 6.25 µM solution of FUN-1. The manufacturer suggests preliminary experiments to optimize the relative concentrations of FUN-1 and yeast cells because excessive loading with FUN-1 can increase the amount of residual green fluorescence, and suboptimal concentration can limit the size and number of the CIVS vacuoles. Optimizing the concentration of FUN-1 can be complicated by the fact that yeast cells process the FUN-1 stain, and more FUN-1 is removed from solution in the presence of dead yeast cells compared to solutions with equal concentrations of live yeast cells. Using epifluorescence microscopy or confocal laser scanning fluorescence microscopy, results can be directly observed to obtain morphological information. Results can also be quantified using flow cytometry or a fluorescence microplate reader. According to the manufacturer, the FUN-1 stain exploits a biochemical process that is highly conserved across yeast and fungi, and thus, the presence of live and dead yeast can be determined in samples containing unknown mixtures of cells.

In summary, vital dyes such as FUN-1 should have wide application in various industrial, agricultural, and biomedical settings. Assessing yeast viability can have great importance in a variety of situations including quality control of commercial yeast products, monitoring the effectiveness of antifungal agents, and data interpretation in biomedical research. ■

References:

1) Hazen KC, Howell SA. 2003. *Candida, Cryptococcus*, and other yeasts of medical importance, p. 1693-1711. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, Fromtling RA (eds). Manual of Clinical Microbiology, 8th ed. American Society for Microbiology Press, Washington, DC.
2) Calderone RA (ed). 2002. Candida and candidiasis. American Society for Microbiology Press, Washington, DC, pp. 451.
3) Stone CW. 1998. Yeast products in the feed industry: A practical guide for feed professionals. http://www.diamondv.com/articles/booklet/booklet/html.
4) Anaissie EJ, Ghannoum M, Marr KM, Pappas PG, Rinaldi MG, Sobel JD, Walsh TJ (eds). Chronic candidiasis. http://www.doctorfungus.org.
5) Millard PJ, Roth BL, Thi H-PT, Yue ST, Haugland RP. 1997. Development of the FUN-1 family of fluorescent probes for vacuole labeling and viability testing of yeasts. Appl Environ Microbiol 63:2897-2905.
6) Product insert: Probes for Yeast Viability, L-7009 LIVE/DEAD Yeast Viability Kit. Revised January 22, 2001. Molecular Probes, Inc., Eugene, OR.

Cutting edge science driving the discovery of new medicine.

Driving the success of GlaxoSmithKline - the world's leading pharmaceutical organization - is a continual search for innovation. Apart from a research and development capability that sets the benchmark for our industry, we're committed to recruiting and retaining the best and brightest by providing unequaled individual and career development opportunities within our organization. Our mission is clear - to prevent, treat and cure diseases, allowing people around the world to do more, feel better and live longer. We currently have opportunities available in our state-of-the-art facility, located in Research Triangle Park, NC.

Manager of Ultrastructural Pathology

Using your technical and intellectual expertise, you will lead the Ultrastructural Pathology Laboratory in producing ultrastructural data. This includes producing high-quality interpretive ultrastructural reports for regulatory submissions; implementing SOP's and ensuring that the laboratory is GLP compliant; collaborating with pathologists, study directors, and project representatives to design ultrastructural pathology components of regulatory and investigative studies; and developing and validating new ultrastructural and morphometric laboratory techniques. You'll also provide consultation and support to other scientific staff, serve as a Project Representative on GSK Project Teams for drug development, and design, conduct and interpret non-clinical toxicity studies.

To qualify, you must have a DVM or PhD, at least 5 years experience in ultrastructural data interpretation, recognition as an expert in transmission electron microscopy (TEM), and extensive experience in a GLP environment, preferably in the pharmaceutical industry. Excellent leadership, project management, decision-making, problem-solving, presentation and oral/written communication skills are essential, as are demonstrated technical knowledge in conducting toxicity studies with laboratory animals and a strong understanding of regulatory guidelines, GLP regulations, mechanistic animal models, and animal welfare legislation.
(Req. ID: 16193)

Electron Microscopy Specialist

In this challenging role, you will fix, process, and examine tissues using transmission electron microcopy techniques in a GLP environment. This includes troubleshooting and correcting technical problems; maintaining the TEM laboratory; analyzing results and taking appropriate next steps; using various image analysis systems to measure and quantify data; ensuring that SOP's (Standard Operating Procedures) and GLP's are followed in all laboratory functions; and researching and introducing new techniques to improve processes.

To qualify, you must have at least 4 years of practical experience in Transmission Electron Microscopy (TEM), including a background in a GLP environment, preferably in the pharmaceutical industry. Strong theoretical scientific knowledge, good technical skills and a working knowledge of laboratory procedures are essential, as are knowledge of GLP practices and experience developing and validating TEM techniques, including immuno-electron microscopy procedures. Background in histotechnology, particularly immunohisto-chemistry, is a definite asset. (Req. ID: 16194)

GlaxoSmithKline is dedicated to supporting you with career-long opportunities and learning. We offer a competitive benefits and compensation package designed to attract and retain the very best. For confidential consideration and efficient processing of your resume, please visit our website at www.gsk.com. Indicating Req ID is essential to search. Principals only, no agencies, please.

GSK is proud to promote an open culture, encouraging people to be themselves and giving their ideas a chance to flourish. GSK is an equal opportunity employer.

Together we can make life better.

gsk GlaxoSmithKline