Viability and Versatility of the Yeast Cell

Michelle J. Henry-Stanley and Carol L. Wells
University of Minnesota, Minneapolis
well002@umn.edu

Yeast is 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 “opportunist 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 coloniza-

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.
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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-1-benzol-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 10⁷ 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:
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