Baker's Yeast and Its Life Cycle

What Are Yeast, Anyway?

Yeast are simple fungi. The term "yeast" refers more to a life-style than to a phylogenetic classification. Yeast refers to the unicellular phase of the life cycles of many different fungi, but it is used more commonly as a generic term for fungi that have only a unicellular phase. The organisms most often called "yeast," such as common baking or brewing yeast, are strains of the species *Saccharomyces cerevisiae* (Fowell 1969a). As fungi, they are classified as ascomycetes, a group which also includes a number of other popular genetic organisms, such as Neurospora and Sordaria (Fincham & Day 1971). Except when we refer to other species of yeast by name, we will use the term "yeast" to refer to *Saccharomyces cerevisiae*.

Yeast have simple nutritional needs. Unable to carry out photosynthesis, they require a reduced carbon source which can be as simple a compound as acetate. In addition, they also require a nitrogen source such as ammonium sulfate. Yeasts can use a variety of organic nitrogen compounds, including urea and various amino acids. The only other complex compound that they require is the vitamin, biotin. Of course, they also require a variety of salts and trace elements.

![Figure 1: Morphology of Yeast Cell Types](image)

A. haploid yeast cells budding  
B. haploid cells forming shmoos and zygotes  
C. zygote budding off diploid  
D. diploid budding  
E. diploid forming asci with ascospores,

Another characteristic of most yeast, including *S. cerevisiae*, is that they divide by budding, rather than by binary fission (Byers 1981). A small bud emerges from the surface of the parent cell and enlarges until it is almost the size of the parent (see Figure 1). While this is happening, the chromosomes of the parent replicate. At mitosis, when the nucleus divides, one of the nuclei is transferred to the bud, and then the two cells separate.

*Schizosaccharomyces pombe*, another popular yeast, is remarkably similar to *S.*
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cerevisiae, except that it grows as cylindrical cells that elongate and split in half by binary fission, much like bacteria. Its cell division is so convenient to observe that this yeast also has potential for use in teaching biology.

The yeast life cycle, like that of all higher organisms, includes a step known as meiosis, where pairs of chromosomes separate to give new combinations of genetic traits.

Ascomycetes, such as baker's yeast, are popular for genetics research because the ascospores they produce in each ascus are the products of meiosis. When yeast are nutritionally stressed, for example by deprivation of either a carbon source or a nitrogen source, diploid yeast will sporulate. The diploid nucleus goes through meiosis, producing four haploid nuclei which are then incorporated into four stress-resistant ascospores, encapsulated in the ascus (see Figure 1). This packaging of the four meiotic products makes genetic analysis particularly simple.

The ease with which baker's yeast can be maintained as either haploid cells or diploid cells is another characteristic that makes them attractive to geneticists.

What Are Yeast Good For?

People have used yeast, undoubtedly one of the earliest domesticated organisms, for controlled fermentation of food and drink and for leavening in baking throughout recorded history. Today, they are also used in a variety of commercial fermentation and biomass conversion processes. Their usefulness is based on their ability to convert sugars and other carbon sources into ethanol in the absence of air (anaerobic), and into carbon dioxide and water in the presence of air (aerobic). Ethanol is a valuable alternative to petroleum as a fuel and as a raw material for the manufacturing of many important commercial chemicals.

Yeast is also good food. It is rich in protein and is an uncommonly good source of the B vitamins. It provides a valuable source of nutrients that are important in low-meat or vegetarian diets. But while few emanations from the kitchen are quite as tantalizing as the yeasty aroma of baking bread, most people agree that pure yeast tastes pretty bad.

Other genera of yeast also have practical uses. Some can use hydrocarbons, such as petroleum, as a carbon source. These organisms can literally convert petroleum into protein. They are being used to remove petroleum as a pollutant from the environment and to convert low-grade hydrocarbons into protein for consumption by animals.

Is There Anything Bad About Yeast?

Yeast can indeed be germs. In fact, one extremely common pathogenic yeast, Candida albicans, is carried by most people in a benign form (Fincham & Day 1971). While in normally
healthy people it is harmless, in those whose immune response is weakened, it can become infectious and turns into a serious pathogen. These infections are particularly hard to control in humans because yeast metabolism is so similar to ours. Drugs that are toxic to the yeast are also toxic to people. Another particularly nasty pathogenic yeast, *Cryptococcus neoformans*, produces a life-threatening meningitis. These yeasts are known as "opportunistic pathogens" because they are a serious threat only to people with impaired immune responses like those who have AIDS.

**How Do Yeast Grow?**

Both haploid and diploid yeast cells divide by budding (see Figure 2). The cell division cycle begins with a single, unbudded cell (Pringle & Hartwell 1981; Byers 1981). This cell buds, the bud grows to nearly the size of the parent cell, the nucleus divides, and the two cells separate into two unbudded cells. The cycle then begins again for both of the cells. The result is an exponential increase in the number of cells with a doubling time equal to the mean cell-division cycle time. This varies with the strain, the growth medium, and the temperature, but can be as short as one hour. At this rate, a single cell can grow into a barely visible colony in one day.

The growth behavior of yeast cultures is similar to that of bacteria. When a growth medium is inoculated, the cells require a period of preparation before they start dividing. Following this lag period, which may be up to several hours, they rapidly enter the exponential phase during which their number and mass double at equal time intervals. After a period of growth at a relatively constant exponential rate, some environmental condition becomes growth limiting so that the rate of increase diminishes and growth eventually stops. The population and mass become constant. The culture remains stationary and the cells remain viable for several hours; if the culture is refrigerated, the cells remain viable for months. Eventually the cells die, and at room temperature or warmer they will undergo autolysis. Their own digestive enzymes become active and they literally digest themselves, reducing their proteins and nucleic acids to their simpler components, while producing a particularly unpleasant stench.

Normal yeast can grow either aerobically in the presence of oxygen or anaerobically in the absence of oxygen. Under aerobic growth conditions, they can support growth by oxidizing simple carbon sources, such as ethanol, acetate or glycerol. If they have adequate oxygen, they will completely oxidize their carbon sources, usually sugars, to carbon dioxide and water. However, under anaerobic conditions, deprived of oxygen, yeast can convert sugars only to carbon dioxide and ethanol, recovering less of the energy. In either case, growth will be limited by some essential nutrient or the accumulation of the toxin.

Yeast grow equally well in liquid media or on a nutrient surface such as an agar plate or an exposed surface of some kind of food. In liquid, they must be stirred or shaken if they are to
remain aerobic; otherwise, they settle to the bottom of the container, consume the dissolved oxygen, and grow anaerobically. On a nutrient surface in a ventilated container, they grow aerobically with each cell forming a visible colony of up to 100 million cells within 2 or 3 days.
FIGURE 2: Cell Division Cycle of *Saccharomyces cerevisiae*
The Yeast Life Cycle: A Complete Sexual Cycle

In sexual organisms, the life cycle (see Figure 3) is composed of a series of events that alternate between a **haploid** phase and a **diploid** phase (Fincham & Day 1971). The transition from the diploid to the haploid phase is the consequence of a specialized cell division -- **meiosis** -- in which the nucleus divides twice following a single replication of the chromosomes. Meiosis yields four haploid nuclei. During meiosis, paired homologous chromosomes in the diploid nucleus interchange parts and are distributed into the haploid nuclei yielding new combinations of genetic traits. In higher organisms a germ-cell line is differentiated from the body (somatic) cells. The germ-cell line produces the reproductive cells that form the **gametes**. The gametes are the cells which carry the genetic information to the next generation while the somatic cells form the rest of the organism and play no direct role in heredity.

**FIGURE 3**: The Life Cycle of Baker's Yeast

The transition from the haploid to the diploid phase results from mating (sexual conjugation) between gametes. This leads to the formation of a **zygote** in which two parental haploid nuclei fuse to form a diploid nucleus. In higher organisms, the gametes are differentiated germ line cells. In higher animals, different gametes are produced by individuals of opposite sex. However, in higher plants, they are produced by different structures, either on the same plant or on different plants.
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In yeast, as in other sexual microorganisms, the complete sexual cycle is present but is much simpler than in higher plants and animals. Permanent differentiation between germ line and somatic cells does not occur. Instead, transient differentiations occur under appropriate conditions to facilitate the essential events. The simplicity of the sexual cycle in yeast and the ease with which all the events can be manipulated and observed provides a unique opportunity for teaching this normally obscure and abstract process through simple, direct, concrete observations.

Sexual Conjugation

Mating in yeast which is mediated by diffusible molecules -- pheromones -- can be readily demonstrated (Manney, Duntze & Betz 1981). When cells of opposite mating type are mixed on the surface of agar growth medium in a petri plate, changes become apparent within two to three hours. As each type of cell secretes its pheromone into the medium, it responds to the one produced by the opposite type (MacKay & Manney 1974). They each respond by differentiating into a specialized functional form, a gamete. The cells stop dividing and change their shape (see Figure 1). They elongate and become pear-shaped. These distinctive cells have been termed "shmoos" because of their resemblance to the mythical but lovable animals in the "Li'l Abner" comic strip. Cells of opposite mating types that are in contact or close proximity join at the surface and fuse together forming a characteristic "peanut" shape with a central constriction -- two shmoos fused at their small ends. The two haploid nuclei within each joined pair fuse into a diploid nucleus, forming a true zygote. The diploid promptly buds at the constriction, forming a characteristic "clover leaf" figure. One can easily observe all of these stages under the microscope.

The mating pheromones that are secreted by haploid cells are small peptide molecules that diffuse through agar (Betz, Manney & Duntze 1981). Consequently, their existence and their effects on cells of the opposite mating types are easy to demonstrate. If cells of the mating type α are grown overnight on agar medium, a high concentration of the α pheromone accumulates in the agar surrounding the growth. Then if cells of the mating type α are placed on this agar, they begin to undergo the "shmoo" transformation within a couple of hours. The effect is quite striking. One can demonstrate the same effect in a liquid medium in which mating type α cells have been grown. In fact, when yeast were first studied yeast biologists used these simple methods to isolate and characterize the pheromones.

Meiosis

Shmoos then, are the gametes in yeast. They differentiate from normal vegetative haploid cells only when a cell of the opposite mating type is present. In a like manner, any diploid cell can go through meiosis forming haploids which have the potential to become
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gametes (Esposito & Klapholz 1981; Fowell 1969b). Meiosis is part of the process of sporulation which is initiated when diploid cells are transferred to a nutritionally unbalanced medium. But the changes become apparent under the microscope only after three to five days when the asci become quite distinctive. Theoretically, all asci should contain four spores but in practice, many contain only two or three. The ascus has a lumpy shape, much like oranges inside a cloth bag. Treating the sporulation mixture with a readily available crude preparation of digestive enzymes from garden snails will remove the wall of the ascus, liberating the spores. When the spores, either within the ascus or after being liberated, are returned to a nutritionally adequate environment, they germinate and undergo vegetative growth in a stable haploid phase. Haploid strains occur in two mating types, called $a$ and $\alpha$ (alpha). Within each ascus, two spores are normally mating-type $a$ and the other two are $\alpha$. When a cell of one mating type encounters one of the other mating type, they initiate a series of events that leads to conjugation. The result is a diploid cell, which grows by mitotic cell division in a stable diploid phase. If one merely transfers a sporulated cell culture to growth medium the result is a mixed population of haploid strains and new diploid strains which are analogous to the progeny from a cross between diploid higher organisms.

Normally, yeast geneticists isolate the spores, either randomly or by micromanipulation, to prevent the haploid strains from mating and forming the next generation. This degree of control and the ability to observe the genetic traits in the haploid phase makes genetic analysis in yeast powerful and efficient. The analysis of random populations of spores while time-consuming, is simple enough for high school students, and is particularly well-suited to individual projects for advanced students.