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Polypore Mushrooms as a Natural Substrate for *Saccharomyces sensu stricto*

Background

Saccharomyces sensu stricto is a complex of yeast species that includes the model organism, *S. cerevisiae*, in various fields of biology and those which are important for industry. However, their ecology and natural history is not well-characterized. Humans have been using yeast for baking and brewing since ancient times, resulting in an evolutionary distinction between strains manipulated for human use and wild strains found in nature. There is a need to know where wild species of *Saccharomyces sensu stricto* can generally be isolated from in nature, and why they are found in certain places, considering they have many uses. For example, wild strains may have beneficial characteristics, such as more favorable fermentation properties. These wild strains may also give scientists insight on phylogenetic relationships with industrial or “domesticated” strains. Previous investigations show that *Saccharomyces sensu stricto* is most frequently isolated from Polypore mushrooms but the reason is not known why. It is possible that the species of *Saccharomyces sensu stricto* can metabolize nutrients from mushrooms and thereby proliferate, resulting in mushrooms being a possible substrate for yeast growth.

Yeast of the genus *Saccharomyces* are single-celled fungi that ferment carbohydrates and produce CO₂ and ethanol as byproducts. Fermentation is a quick and efficient pathway that requires little energy input to break down carbohydrates and harness energy in an environment low in oxygen. They reproduce asexually through budding, or sexually by mating between “a” and “α” types, which is a primitive distinction of sexes [1]. The *Saccharomyces sensu stricto* complex consists of seven species: *S. cerevisiae*, *S. paradoxus*, *S. cariocanus*, *S. bayanus*, *S. mikatae*, *S. kudraivzevii*, and *S. pastorianus*, the latter is a cross between *S. cerevisiae* and *S. bayanus* [2]. These species differ from other species in the

genus *Saccharomyces* because they are vigorous fermenters, tolerate high levels of alcohol, and have a high transformation efficiency [3]. Because of these properties, they are ideal for many uses.

Uses of *Saccharomyces sensu stricto*

Saccharomyces sensu stricto are used in leavening dough, brewing, and winemaking. In baking, a small amount of yeast is incorporated into the dough and baked. The yeast produce gas bubbles of carbon dioxide that give bread its characteristic fluffiness. Brewing and winemaking also require a small amount of yeast to ferment the barley and grapes, respectively, to create the unique taste and alcohol content. Ethanol alone, also, can be harvested from them for use in fuel cells and as an additive in jet fuel and gasoline. As much as 10% of gasoline is ethanol. Also, *S. cerevisiae* serves as a eukaryotic model organism because of its simplicity and ease in handling. They are very small and easy to maintain, they grow quickly (cell division takes about 20 minutes), and because of their high transformation efficiency, foreign genes are easy to incorporate, resulting in easy genetic manipulation. Moreover, all of its DNA is sequenced, and 74% of its genes are characterized, providing scientists with considerable information its function [4].

Although industries already have sufficient strains of yeast, there is always a need for more efficient strains that can cut costs and time. For example, yeast should be able to grow on inexpensive substrates on large scales. Molasses to cultivate yeast is an inexpensive and easy to obtain substrate currently used. They should also be resilient to repeated packaging and storage for transport and sales to brewers and winemakers. Reviving the yeast after packaging should not affect their performance. In winemaking, yeast should be able to produce unique tastes and these tastes depend on their metabolism [5]. In ethanol harvesting, they should generate more ethanol in a given time. New strains of *Saccharomyces sensu stricto* are almost impossible to obtain unless the already existing strains are

genetically modified. There is also an option to find new strains in nature, but there is not enough information on where they can be found in a natural environment.

Biogeography

The geographic distribution of *Saccharomyces sensu stricto* is not well characterized; many of the species have been isolated from the wild in almost all of the continents, but it is not clear whether the species are native to where they were isolated. For example, *S. kudriavzevii* was isolated from Japan [6] but later found in Portugal on oak bark [7]. On the other hand, *S. bayanus* has been found in Russia and Eastern Europe, but nowhere else to date [8]. It is not even clear where in nature *Saccharomyces sensu stricto* members reside. They were first isolated from sugary substrates, such as damaged grapes in a vineyard, hence the name *Saccharomyces*, which comes from the Latin word *saccharum*, meaning sugar. Members of the *Saccharomyces sensu stricto* complex have also been found on complex polymeric carbohydrate sources, such as tree bark, but it is not known if they thrive there or what their role is.

Spatially, yeasts are known to be dispersed by insects, especially fruit flies [9], which are common on mushrooms. Yeast intermixing and diversity is created within the fly's gut, where enzymes break up and recombine chromosomal tetrads from different yeast cells [10]. This mixing is very important and unique to natural strains. Strains used by humans have gone through few natural processes that create genetic diversity. Diversity is usually intentionally created by geneticists by occasional gene splicing and breeding between different species. Because of this difference in natural and human strains, they have evolved differently, posing a new question of evolutionary differences between yeast strains and species.

Previous Research

Madelyn Shapiro, an undergraduate Honors student at UMass Boston, has been investigating where *Saccharomyces sensu stricto* can be found in nature. After sampling fruit, bark, soil, mushrooms, and other possible habitats that have the potential to carry yeast, she found that *Saccharomyces sensu stricto* can be isolated most frequently on mushrooms as compared to other habitats. After analyzing 189 yeast isolates from environmental samples by molecular methods, 54 were positive for *Saccharomyces sensu stricto*, and 32 of those isolates are from mushrooms. Her molecular methods include DNA extraction from each of her isolates of specific fragments from “DNA fingerprint” regions, or regions that are variable according to the specie. This fragment is then copied many times in a process called polymerase chain reaction (PCR). These fragments are then separated according to size (nucleotide base pairs) and electric charge on a gel matrix. Their size and charge are compared to that of the DNA fingerprint regions of the species of *Saccharomyces sensu stricto* to see whether they match. If the fragments match, then the isolate is positive for *Saccharomyces sensu stricto*. Her results reveal that mushrooms most often carry isolates positive *Saccharomyces sensu stricto*, but it is not clear why.

Saccharomyces sensu stricto and Polypore Mushrooms

Mushrooms are also fungi, but multicellular. When comparing mushrooms and a common source of *Saccharomyces sensu stricto*, e.g. grape, they are quite similar in biochemical composition. Aqueous grape extracts consists mainly of glucose and fructose [11], while extracts of *Laetiporus sulphureus*, a representative mushroom, consists mostly of glucose as a monosaccharide [12]. This similarity of composition suggests that *Saccharomyces sensu stricto* can thrive on grapes as well as mushrooms high in glucose and other monosaccharides. Glucose and fructose also happen to be simple, single-unit sugars that are easy to digest. A biochemical difference between mushrooms and grapes is

that mushrooms have more dry content, composed of complex sugars that are harder to break down such as laminaran, fucomannogalactose, and chitin [12].

There is a possibility that yeast may possess enzymes (molecules that facilitate chemical reactions) that break down complex sugars and help break through the tough cell walls of mushrooms. For example, endochitinase has been discovered in *S. cerevisiae* [13]. It resides in the periplasmic spaces, which is located in the periphery of the cell, between the cell wall and inner membrane, poised for secretion [14]. Its only known function is to partially digest the chitinous cell walls between newly budded cells and parent cells so the cells can break free from one another [15], but it is likely that endochitinase can have other functions not yet discovered, such as digesting chitin in mushroom cell walls to reach the monosaccharide food source within the cell, that is, if they are able to use the organic compounds from mushrooms. Similarly, *Saccharomyces sensu stricto* may have other enzymes to help them obtain nutrients in a variety of environments.

From the evidence presented above, I hypothesize that it is possible for the species of *Saccharomyces sensu stricto* to obtain nutrients from mushrooms because of their simple sugar composition and ability to break down chitin with endochitinase. Their relationship seems to be opportunistic because yeasts can grow anywhere where there is a rich sugar source, and they were probably dispersed there by chance.

Future Implications

This investigation can be proven by performing an experiment to test *Saccharomyces sensu stricto* growth in the aqueous phase of polypore mushrooms. This also inspires other experiments such as determining whether the dry phase of mushrooms can improve or deter growth of *Saccharomyces sensu stricto* isolates, and testing the range of mushrooms the isolates can tolerate and whether oak bark and other common locations for isolation can support yeast growth.

Because mushrooms are a potential habitat for *Saccharomyces sensu stricto*, those who are interested in finding natural isolates can start there. These isolates can be tested for specific characteristics for commercial use and their phylogenetic roots and relations can be uncovered.

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