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A literature review of genetically altered yeast and its industrial uses

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ABSTRACT

Yeast is a crucial organism for scientific development and several industries, ranging from food and beverage production to agricultural use. In this literature review, genetic modification of yeast for increased lipid accumulation is discussed in detail, as is nutrient availability and uptake. Finally, agricultural uses and production of brewing yeast (modified and unmodified) are discussed.

Keywords: *Yeast, S. cerevisiae, genetic modification, nutrients availability, industrial use*

INTRODUCTION

Saccharomyces cerevisiae is one of science's most prolific organisms. Its uses range from food and ethanol production to medicine. While it doesn't naturally accumulate large amounts of useful lipids, it can be engineered to do so through the use of genetic modification and modification of the growth culture used. *S. cerevisiae*, as well as other yeast species, can also be manipulated in a number of other ways in order to increase lipid accumulation.

As mentioned above, several yeast species can be used in the food and medicinal industries. Different species can also be put to use in the agricultural and brewing industries with a wide variety of uses and end results. The species used range from specifically bred species to wild type species found in nature.

A positive aspect of yeast is that it is easy, as well as cost efficient, to genetically modify it for any number of wanted effects. One example is the genetic modification of yeast in order to increase lipid production (which isn't something that yeast typically produce or store large quantities of naturally).

There are several ways to go about this modification, but a common one is to simply add plasmids containing the wanted gene to a strain of yeast. This can be done to increase lipid accumulation in the modified *S. cerevisiae* to 2.6 times the control strain (Peng, He, and Haritos, 2017). These plasmids can be procured from plant species (Peng, He, and Haritos, 2017), bacteria (Ghogare, Chen, and Xiong, 2020), or other yeast species.

Nutrient availability from the growth medium can also have quite the effect on yeast, both genetically modified and nongenetically modified. Providing different types of nutrients can increase yeast activity, as well as increasing stress tolerance in some cases. Increased yeast activity can be accomplished by the addition of a wide variety of nutrients, including diammonium phosphate to increase nitrogen availability (Tesniere et. al., 2019) or caffeic acid and caffeic acid phenyl ester (Prudencio et. al., 2019).

Yeast, as mentioned above, also has a basis of performing well in the agricultural industry. There are several of the 1500 species of yeast that can help promote plant growth in a variety of ways, including pathogen suppression and siderophore production (Fu et. al., 2016). Yeast can also synthesize proteins, glycerol, and enzymes that can be utilized in a variety of ways in agricultural circles (Mukherjee et. al., 2020). While genetically modified yeast qualify as GMO's and aren't utilized a lot in the brewing industry, Gibson et. al. (2020) showed that a system called Adaptive Laboratory Evolution (ALE) can be utilized. ALE can be described as guiding a few hundred generations of a yeast strain through evolution in a specific way aimed at producing a desired genotypic or phenotypic trait.

Yeast is a crucial organism to scientific advancement, as well as several industries. This paper will aim to explore the topics discussed in this section in detail, starting with genetic modification, and ending with use in the agricultural and brewing industries.

GENETIC MODIFICATION

Genetic modification can greatly increase levels of lipid accumulation in several types of yeast. However, it is *Saccharomyces cerevisiae* and *Yarrowia lipolytica* that are most commonly used for this. There are several ways to modify greater lipid accumulation, including adding genes, removing genes, or blocking lipid breakdown pathways among the strain of yeast being worked with.

Peng, He, and Haritos (2018) studied the *S. cerevisiae* wild type strain BY4741. Their research team chose to first study how each gene being used would affect lipid levels when expressed individually in the yeast strain. They found that cells with either the Tgl3 key lipase deletion or the addition of the *AtDGAT1* gene addition showed the greater levels of lipid accumulation than the control. This was consistent with previous reports (Peng, He, and Haritos, 2017).

However, Peng, He, and Haritos (2018) also found that the *Ald6-SEACS*^{L641P}*, ACCL**,* and *Atclo1* genes did nothing to improve the lipid content compared to

the control. The authors of the paper did note that the host strains and culture mediums used may have been the cause for the unexpected results from these genetic modifications. Cell growth was also limited (Peng, He, and Haritos, 2018).

Peng, He, and Haritos (2018) engineered two new yeast strains, B1 (wild type strain with *AtDGAT1* gene added) and B2 (wild type strain with *AtDGAT1* and *AtClo1* genes added). B1 and B2 also both had the Tgl3 lipase gene knocked out. The two were very similar in total lipid yields, as well as growth and biomass levels.

The authors showed that modifying genes serving many different purposes could increase lipid accumulation. These purposes included fatty acid biosynthesis, lipid accumulation, and stabilization. There were some difficulties in increasing lipid accumulation, as well as sustaining cell viability. This was addressed through "…a combination of metabolic engineering and bioprocess strategies…" (Peng, He, and Haritos, 2018).

Ghogare, Chen, and Xiong (2020) found thar nonoleaginous yeasts like *S. cerevisiae* far underperform oleaginous yeasts like *Y. lipolytica* in terms of natural ability to accumulate lipids such as triacylglycerols (TAGs). Lipid production in *Y. lipolytica* can be even further improved in a variety of ways, including the deletion of genes involved in beta oxidation, one of the main pathways for lipid breakdown.

Ghogare, Chen, and Xiong (2020) also found that the overexpression of certain target genes was also greatly able to increase lipid production. A shortened version of the *TesA* gene obtained from *E. coli* being inserted into a mutant strain of *Y. lipolytica* was able to increase production from 0.198 g/L fatty acids in the control group to 2.3 g/L fatty acids in the modified sample. The concentration in the modified sample did drop to 1.45 g/L after reaching its peak, but this is still over seven times the amount of fatty acid production in the control group. This was attributed to fatty acid degradation and fatty acids being used for certain biosynthesis pathways after glucose depletion.

The strain of *Y. lipolytica* with the gene *YlAcc1* added also showed significant improvement in lipid production (1.57 g/L and 1.65 g/L fatty acids on days five and seven of the experiment respectively). The strain with the added *YlHxk1* gene only improved to ~1 g/L fatty acids, but the study did note that this genetic modification caused the yeast strain to perform better with fructose as a carbon source as opposed to glucose (Ghogare, Chen, and Xiong, 2020).

The strain of *Y. lipolytica* expressing the *TesA* gene also utilized all of the provided glucose, as opposed to the control groups of two different strains, which still had significant residual glucose at the end of the experimental stage. The reasons behind this aren't certain, but the authors speculated that it was due to rapid conversion of acyl-CoA molecules to their fatty acid counterparts. The strain including the *TesA* gene also showed up to 9.2% more fatty acids by dry cell weight on the 5th day of the experiment (Ghogare, Chen, and Xiong, 2020).

The highest lipid content, as opposed to fatty acid content, still happened in the *TesA* modified strain, but happened on the 7th day of the experiment as opposed to the 5th. It reached 4.64 g/L of lipids. The authors attributed this to rapid turnover between fatty acids and lipids. Lipid content still increased across all modified strains over the parent strain (Ghogare, Chen, and Xiong, 2020).

In their discussion, the authors noted that further studies into the efficiency of this experiment were still needed. This would include finding better pathways for production, bettering the culture used for growth, and helping existing strains of *Y. Lipolytica* to evolve in order to produce more lipids and fatty acids (Ghogare, Chen, and Xiong, 2020).

Shockey et. al. (2011) showed that while the previously discussed studies have implemented genes from non-yeast organisms into yeast species, it is also possible to take a gene from one yeast species and implant it into another. One study found that when the lipase 2 (*LIP2*) gene from *Y. lipolytica* was inserted into *S. cerevisiae*, increased lipase 2 secretion activity was present in the *S. cerevisiae*.

Three different versions of the gene inserted were used. One was simply the *LIP2* gene, one was the gene with additional serine (*LIP2+SER*), and the third was the gene with carboxypeptidase Y from *S. cerevisiae* (*LIP2+CPY*). The *LIP2+SER* showed the most lipase secretion activity, allowing for the highest amount of fatty acid uptake from the medium. The *LIP2* addition allowed for the second most lipase secretion activity, with the *LIP2+CPY* having the least out of the modified strains (Shockey et. al., 2011). Engineering the yeast strain in this way allowed for the uptake of fatty acids from the growth medium, rather than the production of them by the yeast, or simply blocking the pathways that utilize them as sources of energy.

Beopoulos et. al. (2008) showed that the deletion of the *gut2* gene in two different strains of *S. cerevisiae* was able to increase cell growth and lipid accumulation in both strains (Beopoulos et. al., 2008). The study also showed how the growth medium used can increase growth and accumulation. A simple glucose growth medium ended with total fatty acid (TFA) levels below 20% of dry cell weight (DCW) for the wild type (WT) and *gut2* deleted yeast strains. A glucose medium with oleic acid added lead to TFA levels for the WT strain to be ~10-15% DCW and ~30% DCW for the *gut2* modified strain. Using oleic acid that was 98% pure in a glucose-based growth medium showed the most increase in TFA by DCW.

The WT strain had just above 30% DCW, while the *gut2* modified strain had between 50% and 60% TFA by DCW.

These studies collectively show that a variety of oleaginous and nonoleaginous yeast species can be used for lipid accumulation with a lot of success. A variety of genetic modifications ranging from supplementing genes from other species to knocking out pathways that utilize lipids for energy have found success in both types of yeast. Mixing genetic modification of yeast with modification of the growth medium used has also shown to be promising. All in all, the field of using modified yeast for lipid uptake and accumulation is a promising field.

NUTRIENTS AVAILABILITY AND ITS EFFECTS

As noted in the previous section discussing genetic alteration of yeast strains, there are often issues surrounding cell viability after modifications are made. In this case, cell viability refers to each cell's ability to grow and flourish after modification. Therefore, while the afore discussed studies were able to increase lipid accumulation, if cell viability is limited, then the full potential of the experiments fails to be reached. This section of the study will aim to discuss various options for increasing cell viability, and the effects thereof.

Tesniere et. al. (2019) looked at the effects of nitrogen starvation and the repletion of the viability of a wine fermenting strain of Saccharomyces cerevisiae. It was found that almost 4800 genes were affected (either by induction or repression) by changes in nitrogen levels. These genes included genes for transcription, translation, RNA processing, and regulation of metabolic processes.

Tesniere et. al. (2019) used a strain of S. cerevisiae used for commercial wine production. The strain was first grown in a synthetic medium to monitor CO2 levels. It was found that the yeast became nitrogen starved within 42 hours, and 14 grams of CO2 had been produced. The synthetic medium was then changed by the addition of diammonium phosphate (DAP), which provides a source of yeast assimilable nitrogen. Sampling was performed in 15-minute increments for an hour after DAP addition to study the effects.

Tesniere et. al. (2019) found that many of the 4800 genes affected by DAP addition experienced an up-regulation in their use. A large portion of these genes were ones involved in ribosome biogenesis, transcription, translation, and nuclear transport. Also up regulated were genes involved in metabolic processes, regulation of gene expression, and amino and organic acid biosynthetic processes.

There were also many down-regulated genes. These involved genes that played a role in the protein catabolic process, proteolysis, lipid metabolic processes, and glycolytic processes (Tesniere et. al., 2019). If lipid metabolism is able to be down regulated upon relief from nitrogen starvation, which could lead to larger amounts of lipid accumulation. This would allow for more lipids to be produced, and used in medical and industrial processes.

The effects of the study were vast, not only in the number of genes induced or repressed, but in the ways this effected the growth of the yeast. Namely, growth was encouraged, and the general stress response was limited. This is a good step forward in looking at how to manipulate cell viability when needed.

Prudencio et. al. (2019) showed that caffeic acid (CA) and caffeic acid phenethyl ester can also have an effect on cell viability in strains of S. cerevisiae, specifically in the form of antioxidant activity.

The BY4741 strain of S. cerevisiae was used, as well as its mutant strains Δsod1 and Δgsh1. The study aimed in part to find the effects on cell viability of CA and CAPE on cells put under stress by H2O2. Yeast cells were exposed to chlorogenic acid, CA, and CAPE while in the exponential phase for 2 hours before being washed with a K-phosphate buffer. The yeast was then "…resuspended in YPD medium and then incubated with 1.0 mM hydrogen peroxide for 1 h (stress control) were maintained for comparison purposes," (Prudencio et. al., 2019).

In the study done by Prudencio et. al. (2019), all yeast strains treated with chlorogenic acid, CA, and CAPE showed increased cell tolerance to the oxidative stress induced by treatment with H2O2. The control strain BY4741 had CA treatment performing the best, increasing stress tolerance by ~106% compared to cells that weren't exposed to it. "Additionally, both chlorogenic acid and CAPE were effective in comparison with the control strain, obtaining increases in viability of around 76 and 91% respectively," (Prudencio et. al., 2019).

Prudencio et. al. (2019) showed that the Δsod1 and Δgsh1 strains also had good results, with all three treatment options showing improvement over the yeast cells that were only exposed to the H2O2. The Δgsh1 strain, however, performed better than the Δsod1 strain.

This study showed yet another option for increasing stress tolerance and cell viability, not only in a base strain of yeast, but also in genetically mutated strains. Similar studies would have to be performed on yeast strains modified for lipid accumulation, but the results are promising. This could lead to regaining the cell viability lost in strains modified for lipid accumulation.

Wing et. al. (2020) performed a study aimed at studying the effects of a freeze–thaw cycle on the cell viability of several yeast strains. 4 strains were crossed to create the S. cerevisiae strain SGRP-4X. The strains crossed were "…a European wine strain (DBVPG6765); a West African palm wine strain (DBVPG6044); a North American oak tree isolate

(YPS128); and a Japanese sake strain (Y12)," (Wing et. al., 2020).

In the study done by Wing et. al. (2020), growth medium with 15% glycerol and 25% glycerol were used to ensure that any effects found weren't due to differing glycerol levels. It was found that the original S. cerevisiae strains didn't show any significant increases in cell viability, but the SGRP-4X strain showed significant improvement in both the 15% glycerol and 25% glycerol mediums after several freeze – thaw cycles. Further experimentation was done to ensure that the differences in improved cell viability in both mediums wasn't due to the glycerol in the mediums, but in fact due to the freeze – thaw cycles.

Studies would need to be done to see if the same results could be reached in strains of yeast genetically modified for higher lipid accumulation. However, the results of cryopreservation are promising, and using this method would provide a cheap, easy way to restore the lost viability in said strains.

INDUSTRIAL USES

Mukherjee et. al. (2020) showed that yeast, whether genetically modified or not, has several industrial and agricultural uses. "Study shows approximately 1500 yeast species were listed in the latest edition of the book 'The Yeasts: A Taxonomic Study (2011)'" (Mukherjee et. al., 2020). With this many species, there are a variety of uses spanning multiple specific processes among industry and agriculture. These include food and beverage fermentation, glycerol production, enzyme synthesis, protein synthesis, acting at a PGP (plant growth promoting) microbe, and more. Species of yeast applicable in these areas range from *Candida famata* to *Zygosaccharomyces rouxii*.

As an agricultural tool, yeast "can produce some plant growth-promoting enzymes, including indole-3-acetic acid (IAA), phosphate solubilization and reduced growth of plant-pathogen by releasing cell wall degrading enzymes (Fu et al. 2016)," (Mukherjee et. al., 2020).

In the study by Fu et. al. (2016) samples of yeast were gathered from the areas directly surrounding (phyllosphere and rhizosphere) *Drosera spathulata*, a medically significant plant. The samples were tested for their ability to produce a variety of plant growth promoting things, including IAA (indole-3-acetic acid), ammonia, and polyamine. Siderophore activity and fungal cell wall degradation abilities were also measured, as well as the action yeast took against *Glomerella cingulate*, a fungal pathogen.

Fu et. al. (2016) used yeast samples gathered from the leaves of the *D. spathulata*. IAA levels were quantified using Salkowski's reagent. Results showed that in most isolated strains, IAA production levels was dependent on the presence of the amino acid Trp. Strains with lower or nonexistent Trp showed lower levels of IAA production. To measure ammonia production, the yeast isolates were treated with peptone water and incubated for five days before being treated with Nessler's reagent. 13 of the 34 strains showed strong ammonia production, while 21 did not.

Fu et. al. (2016) measured polyamine production by setting the yeast cultures onto a Long Ashton decarboxylase (LAD) agar plate and incubated for nine days. Red rings around the colonies at the end of the incubation period meant that polyamine production was exhibited. Unlike with IAA and ammonia production, only eight of the yeast strains exhibited polyamine production.

Fu et. al. (2016) measured siderophore production by placing yeast isolates on a double layered chrome azurol S (CAS) agar plate, then being incubated for 5 days. Any colonies exhibiting orange rings around them at the end of the incubation period exhibited siderophore production. Siderophores were only produced by 15 of the strains of yeast isolated.

Fu et. al. (2016) measured cell wall degrading enzyme production in a couple different ways, one being growth of yeast cultures on a skim milk agar plate. These plates were the incubated, again for five days, and clear zones on the agar after incubation were indicative of cell wall degrading enzyme production. 25 yeast strains were found to have cell wall degrading enzymatic activity.

Gibson et. al. (2020) wrote that yeast is also the fermenter employed by brewers of beer globally. A genetically modified yeast was actually one of the first GMO's cleared for use in the food industry. However, due to levels of public acceptance for GMO's being used in the food industry, most brewers steer away from such an approach.

Some brewers are turning to the possibility of using wild type yeast strains in production in order to create new, novel products. Recently, a new wild type strain (*Saccharomyces euhayanus*), began to be used in brewing, and was discovered in Patagonia.

S. euhayanus is related to *S. cerevisiae* and *S. pastorianus*, both of which are also used in beer production. "This genetic relatedness inspired studies focusing on its brewing potential. These studies revealed a number of relevant preadaptations in this species, chiefly the ability to use the main brewing sugar maltose, the generation of pleasant aroma profiles, and cold tolerance," (Gibson et. al., 2020).

Yeast strains from other areas of the food industry could also potentially be repurposed as brewer's yeast. These include strains isolated from sourdough cultures (for their ability to utilize maltose), strains isolated from sugarcane rum fermentations (for their high ethanol tolerances), and strains used in probiotic preparations (these would mainly be used for

production of low alcohol beers) (Gibson et. al. 2020). Adaptive Laboratory Evolution (ALE) is beginning to be used instead of genetic modification by traditional means. Instead of splicing in new genes or cutting out unwanted ones, strains of yeast can be introduced to a variety of stimuli in a lab in order to guide them towards evolving and taking on new characteristics. “In addition to genetic diversity, ALE requires a selectable trait. Directly selectable traits are, for example, an increased growth rate, a shortened lag phase, increased viability, improved substrate consumption, prototrophy for certain nutrients, buoyancy, flocculation, and sedimentation. ALE becomes more challenging if the phenotype to be improved is not directly selectable,” (Gibson et.al., 2020).

Gibson et. al. (2020) wrote that with yeast cultivation, serial batch transfers are commonly used during ALE. Cultures are grown in test tubes, and during exponential growth, some of the culture is transferred to a different test tube. This is done to be able to select the best yeast cells to continue the chain of evolution. Serial batch transfers are sometimes done in bioreactors, so that parameters influencing evolution can be controlled, and selected for.

ALE experiments can also be run as continuous cultures in bioreactors, where the parameters can again be controlled and selected for. However they are run, ALE experiments are typically not run for longer than a few hundred generations of yeast so that genome sequencing can be run on the youngest yeast cells. This is done to ensure that the desired affect is done (Gibson et. al., 2020).

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