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Review Article

Saccharomyces Cerevisiae and Its Biotechnological Applications

Abdullah M Al Falih*

Department of Botany and Microbiology, College of Science, King Saud University, Saudi Arabia

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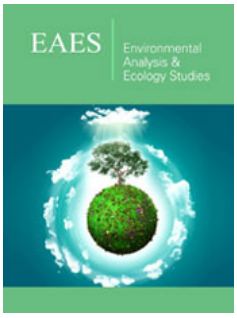

***Corresponding author:** Abdullah M Al-Falih, Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box. 2455 Riyadh 11451 Saudi Arabia

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Abstract

The review has been extensively on the biological activities of yeast, focusing on the various uses of renewable feed stocks and their conversions into fuels and chemicals to cope with the increasing demand on sustainability issues around the world. *Saccharomyces cerevisiae* is the best studied eukaryote and a valuable tool for most aspects of basic research on eukaryotic organisms. *S. cerevisiae* is one of the most popular cell factories and has been successfully used in the modern biotechnology industry to produce a variety of substances such as ethanol, organic acids, amino acids, enzymes and therapeutic proteins. This review will focus on how to use different sustainable solutions to overcome the different environmental impacts on yeast. With a wide range of current developments and future prospects in yeast biotechnology explored and its applications and potentials discussed in general.

Keywords: Yeast, Fermentation, Biotechnology, Industry and Saccharomyces Cerevisiae

Introduction

People have used yeasts and other microorganisms to produce many foods and drinks since ancient times. Bread is a result of the microbial fermentation of the sugars to produce carbon dioxide, which is released into the dough making the bread puffy. The human interest and attempt to benefit from the activities of microorganisms dates back to prehistoric times, but these activities were not used in industry until around the middle of the twentieth century as a result of the cooperation that arose between microbiologists, engineers and capital owners to develop industries based on the use of Microorganisms. Microbes are also essential in the production of beer and wine, as the sugars are converted into alcohol. Microbial fermentation is also a step during the chemical process of recycling waste. The raw materials used in the industry vary and the most important of which are the following: corn steeper extract. barley, cellulose, starch, grab, alcoholic products, soybean seeds, hydrocarbons and sucrose [1].

Figure 1 illustrates a general approach whereby metabolic engineering within a systems biology framework can play a vital role by using genetic modification to build the strain with the help of the metabolic model approach and further improve it by fermentation to obtain all growth materials as analysis of different omics will help further to obtain better insights for the model refinement.

Baker's Yeast Biotechnology

Baker's yeast has been used since ancient times since people knew bread and made it an essential component of their food. Certain strains of *Saccharomyces cerevisiae* are now used to improve the taste, consistency and texture of the dough. This yeast breaks down (fermentation) the sugars, resulting in alcohol and CO_2 bubbles that remain trapped in the dough, which increases the size of the dough and inflates it. Usually, an amount of yeast is added to the mixture of flour and water, an amount of salt and some sugar, then the dough is left to ferment at a temperature of about 25 °C. During this period, the yeast breaks down the sugars in the dough into a mixture of alcohol and carbon dioxide. Carbon dioxide gas in the bread, which gives the bread its distinctive texture. Humans have resorted to the use of yeasts in many matters, such as the production of antibiotics to treat diseases caused by pathogenic microorganisms, as well as the production of many important food or industrial materials such as bread, dairy products, pickles, vitamins, enzymes, acids, proteins, fats and steroid of vehicles, as happened with the Germans in World War II.

Baker's yeast is simply beer yeast produced via a submerged fermentation process carried out in the presence of oxygen (Figure 2). Aerobic conditions favor yeast cell production, which is not of interest to ethanol producers, but is important when a large amount of cell mass must be produced.

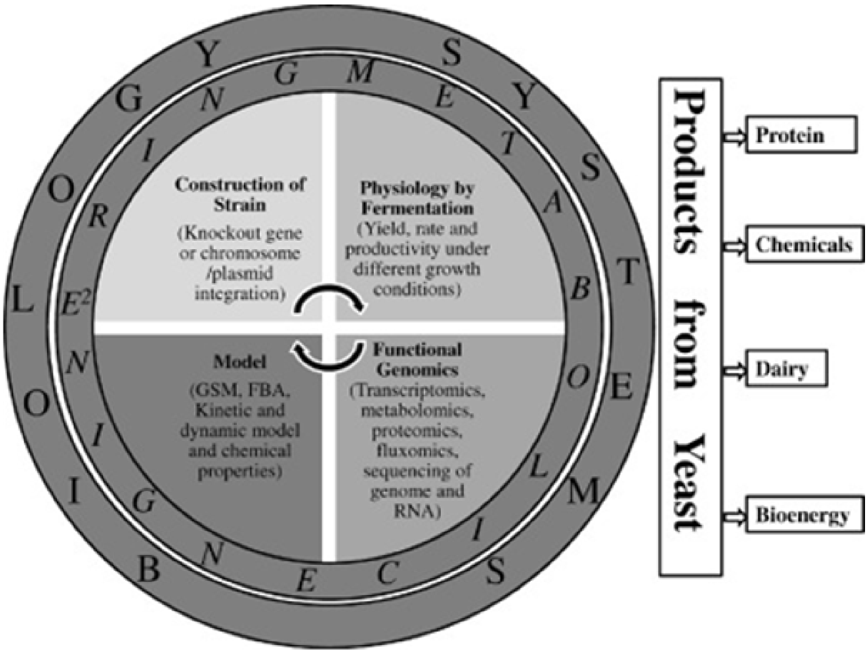

Figure 1: A general metabolic engineering approach for different industrial productions (Nandy and Srivastava 2018).

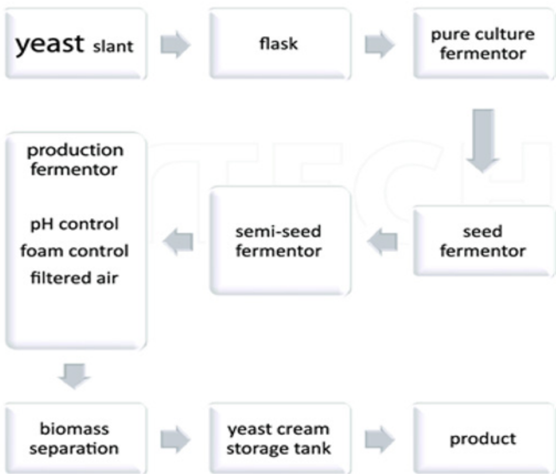

Figure 2: Baker's yeast production process.

Some sugars are directly available to the yeast, as the flour itself contains about 2% of the available sugars in addition to the sugars that are added, such as sucrose and cane sugar, and then substituted for the sugars from wheat grain starch by two types of enzymes, alpha-amylase and-amylase. Which is one of the basic ingredients of the flour and is activated by water and the beta amylase splits the starch from the ends into two units of glycose to give maltose, which is a double sugar, and at the same time the alpha-amylase breaks down the long chain from the inside to separate it into several short chains, giving the beta-amylase the opportunity to act on it, resulting in a mixture of maltose and glucose. Yeast converts glucose into alcohol and carbon dioxide. Maltose is often fermented by yeast at the end of the fermentation process when all other sugars are consumed. Disaccharides such as sucrose and maltose are degraded by hydrolytic enzymes in the cell into monosaccharides. Although some types of yeast can grow on starch, they are less effective than other types that grow on monosaccharides and disaccharides [2,3].

Although the main function of yeast is to increase the size of the dough, it has some other effects [4].

In the dry yeast manufactured by the old methods, a sugar solution must be added and kept at a temperature of 26-32 °C for 6-12 hours until the cells become active in number large enough to carry out the fermentation process. As for the yeast manufactured by modern methods, it can be used directly without the need for previous treatment.

It should be noted that the global production of baker's yeast reaches more than two million tons annually, and it is present in several forms, including pressed yeast, active dry and instantaneous dry, and these pictures differ from each other in the degree of activity and the extent of stability [5].

Beverage and Food Biotechnology

In the European countries yeast biotechnology, a one million tonnes is produced annually, and about 30% of which is exported globally. The worled market's annual growth rate was 8.8% from 2013 to 2018. *S. cerevisiae* has been an essential component of human life because of its extensive use in food and beverage fermentation industry in which it has a high commercial significance. A discussion on the contribution of *S. cerevisiae* in wine, bread and cocoa fermentations follows, highlighting approach such as the process of biochemical reactions that take place in the yeast cell and whose products determine the terminal products, the traits that strains should have in order to be successful starters and the potential of exploiting native strains in industry. In recent years, there is an alternative vinifications employing mixed yeast inocula of *S. cerevisiae* and non-*Saccharomyces species* as cultural fermenter starters. So several researchers and many biotechnological industries have turned to vinification processes involving fermentations with mixed yeast inocula. As non-Saccharomyces inocula have served member species of several genera as shown in Table 1, among them Candida, *Debaryomyces Hanseniaspora, Issatchenkia, Metschnikowia, Pichia, Kluyveromyces/Lanchancea, Torulaspora, Wickerhamomyces*. The mixed starters of these yeasts are applied in a co-, or sequential fashion and often in varying ratios of cell numbers.

Table 1: Mixed starter cultures of S. cerevisiae with non-Saccharomyces species leading to improved organoleptic traits [22].

Inocula Composition	Major Achievement/Negative Results	Fermented Material	Mode Of Inoculation
S. cerevisiae-Candida sake	high levels of esters and fatty acids enzymatic activities	Grape must	Co-inoculation
S. cerevisiae-Debaryomyces vanrijiae	Increased levels of terpenes and higher alcohols	Grape must	Co-inoculation
S. cerevisiae-Hanseniaspora uvarum	Increased production of volatile compounds, esters and terpenes	Grape must	Co- and sequential inoculation
S. cerevisiae-Hanseniaspora vineae	Two-fold increase of the concentration of 2-phenylethyl acetate in the sequential inoculation	Grape must	Co- and sequential inoculation
S. cerevisiae-Hanseniaspora vineae	Increased production of acetate esters and some ethyl esters, decreased production of higher alcohols and some medium chain fatty acids	Grape must	Sequential inoculation
S. cerevisiae-Issatchenkia orientalis	Reduction of malic acid	Grape must	Co-inoculation
S. cerevisiae-Lanchancea thermotolerance	Reduction of pH, increase of 2-phenylethanol and glycerol	Grape must	Co- and sequential inoculation
S. cerevisiae-Metschnikowia pulcherrima	Increased production of polysaccharides, glycerol and volatile compounds. Reduction of volatile acidity.	Grape must	Co-inoculation
S. cerevisiae-Metschnikowia pulcherrima	Increased glycerol concentration, reduction of volatile acidity and total acidity	Mango pulp	Co-inoculation
S. cerevisiae-Metschnikowia pulcherrima var. zitsae	Improved aromatic bouquet	Grape must	Sequential inoculation

S. cerevisiae-Pichia guilliermondii	(Negative outcome) Production of taste spoiling phenol compounds	Grape must	Sequential inoculation
S. cerevisiae - Torulaspora delbruckii	Improved aroma	Amarone	Co-inculation
		Must	
S. cerevisiae - Torulaspora delbruckii	Increase of ester production	Grape must	Co-inculation
S. cerevisiae - Torulaspora delbruckii	Increased glycerol concentration, reduction of volatile acidity and total acidity	Mango pulp	Co-inoculation
S cerevisiae - Wickerhamomyces anomalus	Increased levels acetates, ethyl esters and lineal alcohols. Reduced levels of organic acids	Grape must	Sequential inoculation

Chemical Production Biotechnology

Saccharomyces cerevisiae is widely applied in microbial biotechnology production of chemicals, metabolites via genetic manipulation which is relatively easy and experiences from its wide use in the existing industrial fermentations that might directly benefit the yeast *S. cerevisiae* processes [6]. The genetic engineering strain of *S. cerevisiae* via metabolic activities under different metabolism studied for production of various chemicals of biotechnological industry. While talking about chemical product, the ethanol should come first which is producing naturally from *S. cerevisiae*. This fermentation process is illustrated the production of ethanol from yeast fermentation where glucose is used as substrate where one mole of glucose (180g) is converted to two moles of ethanol (92g) plus two mole of carbon-dioxide (88g) and it produces energy (26.4 Kcal) from this reaction [7]. Ethanol or related biofuel or bioenergy will discuss in the last part of this paper.

Yeast can also be considered as an alternative source of fats. Some species are able to synthesize and accumulate more than 20% of the biomass in the form of neutral fats and for this reason it is called oleaginous yeast. Under conditions of optimal growth or as a result of genetic improvement, the level of fat accumulation can reach up to 70%. Oily yeast contains types such as, *Saccharomycopsis, Pichia (Hansenula), Lipomyces, Pseudozyma, Rhodosporidium, Rhodotorula, Trichosporon, Trigonopsis and Yarrowia* [8].

Excretion Products

To obtain excretion products, yeasts are grown on a carbon source such as molasses, and molasses represents the lowest degree of purity on which sucrose is found, and molasses, besides sucrose, contain reduced sugars, mineral salts, organic materials and water, and it is considered a residue of the sugar industry, whether from cane or sugar beet or corn. Many industries depend on molasses, such as the manufacture of various alcohols, yeast and organic acids. The chemical composition and natural properties of molasses varies according to several things, including the type of sugar cane or beet used in industry, climatic factors during the planting season, agricultural factors in terms of soil type, fertilization, and so on. Among the most prominent substances or products excreted from yeast cells during the fermentation process, we mention the production of Ethanol Glycerol as follows [9].

Production of Ethyl Alcohol

It is worth noting that there are many factors that should be controlled when producing ethyl alcohol. In the forefront comes to make sure of the strain used and its purity. The fermentation process is carried out by *Saccharomyces cerivisae* and must be highly efficient in the production of alcohol and carbon dioxide, and its ability to withstand high concentrations in the fermented sugar and alcohol [10]. The initiator is prepared from the preserved pure select strain, and several successive activations are carried out from this culture in a sterile fermentation solution at a temperature of 25-30 °C until cells are sufficient to inoculate 4 liters of the nutrient medium, then the inoculation steps are transferred from the laboratory to tanks [11,12].

The sugar concentration used in this industry ranges between 10-18% and the usual concentration is approximately 12%. It is known that Molasses contains most of the nutrients needed for fermentation. However, ammonium salts in the form of sulfate or ammonium phosphate are sometimes added to the fermentation solution as a source of nitrogen and phosphorous [13].

Production of Glycerol

Glycerin is used in the manufacture of medicines and beverages and in the manufacture of paints and cosmetics. The German researcher Newporg published his research on the fermentation that he observed when adding sodium sulfite to alcoholic fermentation by the yeast. Note that the first observation of glycerin production during the fermentation process was recorded by Paster, where it was observed that yeast formed glycerin at a ratio between 2.5-3.5% of the fermented sugar. The formation of glycerin depends on the diversion of the alcoholic fermentation process by withdrawing acetaldehyde when it is formed, so it becomes unavailable for oxidation of NADH2, which in this case is used to reduce P-3 - glyceraldehydes. The oxidation of NADH2, or by making the fermentation medium alkaline, leads to directing NADH2 in the reaction to reduction. P-3 - glyceraldehydes is commonly used in the production of *Saccharomyces cerevisiae* glycerin [14].

With glycerine, ethyl alcohol and acetaldehyde are synthetically presented with only 20-25% glycerin, and the alcohol and acetaldehyde are separated by distillation. Note that glycerin production is increased by the use of acids and alkalis or their salts.

There are several known methods for producing glycerin by fermentation, and in all the methods they use a basic environment that contains fermentable sugar, to which the necessary salts and nutrients are added, and then pollinate the environment with a culture of yeast and keep it at a temperature of 30-37 °C to form additives of the year. The first method produced by the Germans, in which sodium sulfite is added to the fermenting liquid, usually the acetyldehyde is reduced to ethyl alcohol, but with the addition of sodium sulfite, it reacts with the acetaldehyde and turns it into a stable compound that is not amenable to receiving hydrogen-hydroaldehyde. To reduce the acetaldehyde to ethanol it goes to the reduction of the glyceraldehyde-3-phosphate fraction to glycerin [15].

$$CH_3CHO + NaHSO_3 + H_2O \rightarrow CH_2OHCHO$$

Glycerin is then obtained by distillation and then purified by distillation under a certain pressure.

As for the second method for producing glycerin, it is known by the American method, in which an environment of sugars is fertilized with the selected strain of yeast and incubated at the appropriate temperature, and a substance that gives an alkaline effect, such as sodium carbogamate, is added gradually according to the speed of its disappearance. Ethyl This reaction is known as the Cannizzro reaction and the duration of fermentation ranges from five days to a week.

$$NaCO3 + H_2O \rightarrow NaOH + HCO_3$$

$$2CH_3CH \rightarrow CH_3CH_2OH + CH_3COOH$$

Compounds Made by Special Reactions

In nutritional biotechnology, the "phenotype" should be considered a fundamental characteristic when developing methods for selecting yeast. Functional traits are what matters most in a particular ecosystem when looking at the mechanisms of general microbiology. Previous studies have been extensively researched over the past decades through specialized research on the chemical determination of compounds derived from the metabolic activity of yeasts. From these studies it can be concluded that many Fermentation products, including ethyl and acetate esters, Higher alcohols, Fatty acids, Lactones and Sulfur compounds [16]. Among the materials produced by special reactions, compounds made by special reactions, which are popular in yeast cell reactions, we mention Ephedrine, as well as hydrocarbon derivatives, in addition to what are known as radioactive biochemical [17].

Dairy Products Biotechnology

One of dairy products is cheese, that is found usually in most complex nature, fascinating, and diverse foods. Some strains of yeast culture which are Kluyveromyces lactis and *Debaryomyces hansenii* have been reported actively grown on soft-cheese model curds [18,19] by taking a month periods for the ripening. Also, kefiris reported as fermented milk product beverage, which is produced by fermenting grains of the kefir through symbiotic interaction of bacteria and yeasts exist in kefir grains. Some yeast strains have been reported such as *K. marxianus, Candida kefyr, D. hansenii and K. lactis var*. Kefir has shown the therapeutic characters as a natural product beverage. These yeasts are actively involved in development of favorable kefirsensory properties [18].

Other dairy product, koumiss is slightly alcoholic yeast fermented mare's milk beverage. Koumiss is produced by applying a natural mixed starter of lactic acid bacteria and a strain of yeasts. It is processing for aroma, texture, as well as the nutrients beneficial to people health is done by using *Saccharomyces cerevisiae* and other yeast strains such as *Candida pararugosa, Dekkera anomala, Geotrichum sp., Issatchenkia or-ientalis, Pichia deserti-cola, P. fermentans, P. manshurica, P. membranaefaciens, Kazachstania unispora*, and *Kluyveromyces marxianus* [20]. Acidophilus- yeast fermented milk has found to vary from Indian Dahlmainly in kind of microbes involved, flavor, body texture, consistency, antibacterial activity and chemical composition. In this beverage product the yeast strains such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* has been added to put the antioxidant characteristics of fermented milk and enhance the viability of bacterial strain. This beverage product has a lactic acid content (1·5 to 2.0%) that help in cure of human stomach disorder [21].

Yeast and Biofuels Biotechnology

Saccharomyces cerevisiae yeast strains have shown cell factory nature [22,23]. Many genetically modified or metabolic engineering ome strains of *Saccharomyces cerevisiae* have been turned into a producer of higher alcohols such as 1-butanol and iso-butanol, farnesene, bisabolene, and biodiesel, Bioethanol is alcoholic beverage and two carbon organic compounds, produced by genetic engineered strain S cerevisiae ATCC 20602 in 7.5-l vessel, that having glucose media, operated in fed-batch mode [24].

In an aerobic fermentation condition, ethanol concentration (20g l-1) was recorded while in an anaerobic system, ethanol concentration was reported 85g l-1 in 70 h with higher cell viability (88%). 1-Propanol is a primary alcohol with three carbon compounds. It has high numbers of octane and suitable for engine fuel use. While production of propanol has been reported too expensive to be a common engine fuel. So, suitable microorganism strain should be selected for biosynthesis of 1-propanol. The yeast strain influence has found to be very strong forn-propanol and less evident for the production of other alcohols. Increased production of n-Propanolin yeast strains has recorded due to having an impaired ability for formation of hydrogen sulfide [7].

Saccharomyces cerevisiae has been found to utilize not only glucose but also xylose as sugar component. Now a days researchers are trying to develop a modified engineered strain of the yeast *Saccharomyces cerevisiae* which can have 2-Keto acid decarboxylase and alcohol/aldehyde dehydrogenase in metabolic engineering and can develop 2-ketobutyrate compound to generat more quantity of 1-Propanol. Isopropanol or dimethyl carbinol is a flammable chemical with a strong odor and colorless. It has a wide industrial application and household usages, also involved in an ingredient of some chemicals such as detergents, antiseptics and disinfectants [25,26].

Conclusion

The yeast *Saccharomyces cerevisiae* is shown to be the best studied microorganism and one of the common widely used eukaryotes in a variety of industrial production processes, such as bread, ethanol, beverage and food biotechnology. Optimization of pathway and construction can be achieved by using metabolic engineering with revolutionized next generation sequencing and together improve the DNA synthesis technique [27]. This review, describe the combination of both systems biology, biotechnology and attraction metabolic engineering. Also known as synthetic biology as a powerful framework to elaborate various industrial applications consisting mainly pharmaceuticals, chemical, dairy and biofuel.

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