S. cerevisiae Bio-Ethanol Production as a Sustainable Energy Source

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ABSTRACT

We rely on fossil fuels, which will be outdated in a few decades. Many people are looking for solutions to the current energy dilemma in renewable energy, notably biofuels. Among the various biofuels, bioethanol looks to be the most cost- So Saccharomyces cerevisiae is a well-known bioethanol producer. Yeast cells are stressed and inhibited during fermentation, limiting their efficiency for commercial bioethanol generation. Adopt alternative signal transduction mechanisms to defeat these yeast cells. This analysis focuses on common and underutilised carbon feedstocks that can be easily transformed into bioethanol. The several types of protectants, genes, and processes that may be used to design yeast strains are addressed. As a result, we've proposed techniques for using this profitable option for long-term bioethanol production.

Keywords- *S. cerevisiae*, Bio-Ethanol, fossil fuels, renewable energy, biofuels

I. INTRODUCTION

The human population has exploded in recent decades, putting a strain on scarce fossil fuel supplies. 90 percent of global energy demand is met by fossil fuels, coal, natural gas, and oils. However, fossil fuels are finite and contribute significantly to greenhouse gas emissions. Renewable energy is an option, and among the available resources, biofuels seem to be a cost-effective and long-term solution. Sugar crops are used to make current biofuels for bioethanol and biodiesel manufacturing. However, the food vs fuel conundrum puts its long-term use in jeopardy. Other biofuel sources include "non-food crops (such as switchgrass, poplar, and willow), algae, and genetically modified organisms (GMOs). Yeast (Saccharomyces cerevisiae) has long been thought to be the best microbe for ethanol production". It can produce bioethanol from a variety of feedstocks, which are detailed below.

Bioethanol is made from carbohydrates found in starch, cellulose, and hemicellulose. Sugarcane juices, molasses, and maize are the most common bioethanol feedstocks utilised across the globe (Wilkie, 2000). Corn, barley, wheat, rye, potato, sorghum, and cassava are all sources of starch, a polysaccharide of glucose. Starch-containing feedstock must first be transformed to sugar or dextrin through an enzymatic process, with amylase being the most often utilised enzyme. Saccharification converts other complex sugars to simple sugars, which are then fermented to ethanol (Naik, 2010).

'First generation biofuel' refers to biofuel made from carbohydrates, sugars, animal fats, and vegetable oils. However, the food and fuel crisis jeopardises large-scale commercial manufacturing.

Global population is predicted to reach over 9 billion in the future decades, with roughly 2.5 billion additional people added by 2050 (Godfray et al., 2010), posing a threat to the sustainability of food crops for biofuel production. Furthermore, a scarcity of these crops hinders their long-term use and commercialization. Lignocellulose feedstock, often known as "second generation biofuel," may be utilised as an alternative (Kumar, 2009). "Agricultural waste (rice, wheat, maize, and sugarcane bagasse), nonfood plants like poplar, napiergrass, switches grass, paper waste, agro-industrial waste, water hyacinth", and sawdust are all examples of lignocellulosic waste (Yasuda et al., 2014). It seems to be a sustainable energy resource since it is non-food crops.

II. BIOETHANOL FROM AGRICULTURAL WASTE MATERIALS

Food crops such as maize, wheat, and sugarcane are the most common. As a result, an adequate supply of these crops for fuel remains a key barrier to bioethanol production (Cheng, 2011). Corn, wheat, and rice straws, as well as sugarcane bagasse, are the most common agricultural wastes. These waste materials have little nutritional value and are readily accessible and inexpensive. Furthermore, it eliminates the need for separate agricultural land, water, fertilisers, and energy sources. The majority of agricultural waste products are either composted in the field or burned in the fields. These wastes may be utilised as biomass for bioethanol production instead of being disposed of or burned. Other feedstocks, such as vegetable or fruit processing wastes, may be utilised to make bioethanol or biodiesel.

Fermentation Stress Tolerance Mechanism

Its excellent conversion of carbohydrates to ethanol, the yeast S. cerevisiae is extensively utilised in the ethanol production sector (Fig. 1). During fermentation, however, it is subjected to a variety of stressors. 'Fermentation Stress Tolerance'(FST) refers to a set of stress situations and an adaptive mechanism for dealing with them.

Tolerance to Ethanol

S. cerevisiae converts sugar, starch, and lignocellulose to ethanol, but when the amount of ethanol reaches a certain level, it limits development, causes mitochondrial loss, and finally kills the yeast cells (Bai et al., 2004; Ibeas and Jimenez, 1997). Increased ethanol levels influence membrane stability, protein damage, and cell membrane destruction. The primary mechanisms and genes involved in ethanol stress tolerance have been identified in various research. When fed with monounsaturated fatty acids, the knockout strains generated by You et al. displayed tolerance to ethanol (You KM, 2003). Strains lacking ergo sterol were susceptible to a modest dose of intracellular ethanol, according to Inoue et al. (2000) (Inoue et al., 2000).
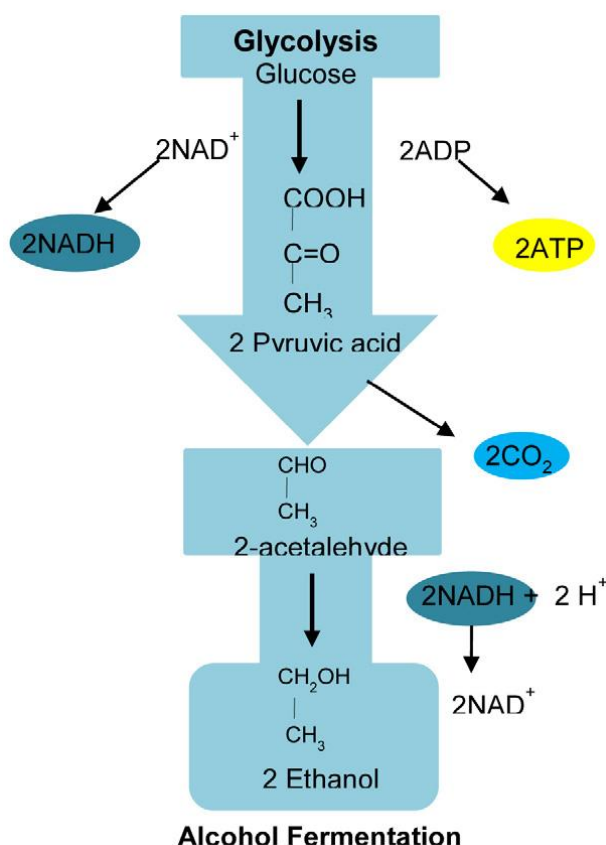

Figure 1: Ethanol fermentation (Inoue *et al.,* 2000).

During glycolysis, one molecule of glucose ($C_6H_{12}O_6$) is transformed into two molecules of pyruvic acid ($C_3H_4O_3$), which is then decarboxylated to yield acetaldehyde (CH_3CHO) and converted to ethanol (C_2H_5OH). Two molecules of ATP are gained and four molecules of carbon dioxide throughout the process (CO_2) to generate ethanol.

Yeast strains that overexpressed genes involved for arginine production, such as ARG4 and CAR1, were shown to preserve cell wall and membrane integrity. In

addition, compared to WT, overexpression of RPI1 enhances ethanol tolerance by almost 50 times. Puria et al. (2009) found that RPI1 overexpression strains are extremely resistant to the cell wall lytic enzyme Zymolyase, indicating that RPI1 may promote cell viability by strengthening the yeast cell wall.

Ethanol denatures functional proteins and proteins in the cell membrane, in addition to damaging the plasma membrane. Cells have evolved adaptive stress tolerance systems in order to endure various external disturbances and preserve internal steady state homeostasis. To trigger a stress response, these biological responses cause changes in gene expression and need signal transduction pathways to communicate from sensors on the cell surface or cytoplasm to transcriptional machinery in the nucleus (Fig. 2).
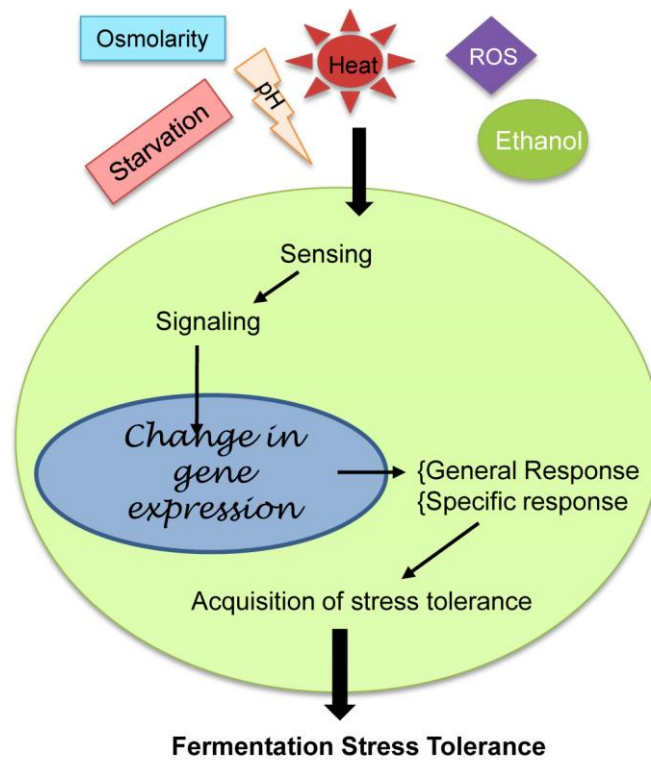

Figure 2: Fermentation Stress Tolerance Mechanism.

In Figure 2 Mechanism of Fermentation Stress Tolerance: During fermentation, yeast cells are exposed to a variety of stressors, including high initial substrate concentrations, nutritional deprivation, progressive buildup of ethanol, temperature increase in the fermentation medium, pH drop, and production of reactive oxygen species (ROS). Under general or stress-responsive circumstances, the cell senses these signals through cell surface or intracellular receptors and transduces the signals for production of specific genes. Fermentation stress tolerance is the result of the production of protective genes or detoxifiers in response to stress.

Ethanol Toxicity in Yeast

The cell wall of S. cerevisiae is constituted mostly of polysaccharides, with only around 15% of the cell made up of proteins. Among the cell wall's principal functions are the maintenance of osmotic balance, the protection of cells from physical injury, and the function as a scaffold for glycoproteins. When exposed to ethanol stress, the primary targets are the yeast plasma membrane, which includes both hydrophilic and hydrophobic proteins. As a result, ethanol has an effect on the structure and function of cell membranes. Ethanol also denatures a variety of plasma membrane proteins. Endocytosis across the plasma membrane is inhibited by ethanol concentrations of 2-6 percent (Lucero et al., 2000). Ethanol inhibits the proton motive force, which is responsible for pumping protons across the plasma membrane. When yeast cells are exposed to ethanol, the activity of the Pma1 membrane protein, which functions as an H-AT Pase, is increased significantly, which is required to maintain intracellular pH and membrane potential, is affected.

III. GENETIC ENGINEERING TO IMPROVE YEAST STRAIN

C_6 and C_5 Carbon Substrate

Yeast has a high affinity for glucose and a low affinity for other carbon sources like galactose. Furthermore, in the presence of glucose, a mechanism

known as 'glucose repression' suppresses the expression of other metabolic genes (Le Borgne, 2011). Various genetically altered *S. cerevisiae* strains have been generated in this area. Overexpression of the genes encoding phosphoglucomutase and positive regulator of Gal4p improved galactose uptake and ethanol production (Ostergaard et al., 2000). Overexpression of the shortened TUP1 gene, which encodes a transcription repressor, increased fermentation rates. In addition, the lactose in whey may be utilised to make ethanol. However, the S. cerevisiae strain usually utilised in commercial ethanol production is unable to metabolise lactose.

Kluyveromyces fragilis (Guimaraes et al., 2008) or genetically altered S. cerevisiae may be employed to metabolise lactose. Starch-rich materials are inexpensive and plentiful, and they may be utilised as bioethanol feedstock. Yeasts with greater xylose fermentation rates, such as Pichia stipitis and Pachysolen tannophilus, may be utilised to ferment five carbon sources, including xylose (Jeffries, 1985; Jeffries et al., 2007). This conversion may be accomplished by the enzymes xylose isomerase, xylose reductase, and xylitol dehydrogenase, among others (Klimacek et al., 2014). After being converted to xylose, xylulose is phosphorylated to become xylulose-5-phosphate, which is then metabolised to form ethanol. Klimacek et al. (2014) created an evolutionarily designed S. cerevisiae strain (IBB10B05) that converts xylose to ethanol effectively used endogenous xylose digesting genes coding for sorbitol dehydrogenase, aldose reductase, and xylulose kinase to ferment xylose to ethanol in a genetically engineered strain of S. cerevisiae (Konishi et al., 2015). Apart from xylose, yeast does not use arabinose for cost-effective ethanol synthesis from lignocellulose feedstock; it must be channelized for ethanol production. Genetically engineered strain of Saccharomyces cerevisiae that expresses the "araA, araB, and araD genes from the bacterium Lactobacillus Saccharomyces cerevisiae Bio-ethanol Production, A Sustainable Energy Alternative S205 plantarum to anaerobically digest arabinose and convert it to ethanol".

IV. COMMERCIALIZATION AND FUTURE PROSPECTS

As the world's population grows, so does global energy consumption. Biofuels seem to be a cost-effective and long-term energy source. However, biofuel commercialization is still in its early stages. Some of the hurdles for commercialization include the initial cost of investment, the lack of arable land, and the seasonal nature of agricultural commodities. Algae is the ideal alternative in this case since it can flourish and grow abundantly on non-arable terrain ranging from wasteland to aquatic ponds. The lignin component of algal cell walls is low, and internal starch granules can be easily converted to ethanol (Han et al., 2015). To achieve cost-effective ethanol production from algae biomass, it is

critical that all of the carbohydrate content of the algal feedstock be converted to ethanol at 100 percent conversion efficiency. In a single bioreactor, simultaneous saccharification and fermentation (SSF) may be accomplished, lowering fermentation costs by reducing the quantity of equipment required. If nations throughout the globe need to become self-sufficient and minimise crude oil imports, research should concentrate on improving harvesting and oil extraction techniques, as well as boosting biofuel crop biomass. Genetic, molecular, and eventually synthetic biology approaches can alleviate all of these problems (Lee, 2010).

V. CONCLUSION

Finally, in light of the near-term characteristic of renewable energy and demand and supply, additional possibilities must be explored, therefore diversifying the sources. 'First generation biofuel' refers to biofuel made from carbohydrates, sugars, animal fats, and vegetable oils. Sugarcane juices, molasses, and maize are the most common bioethanol feedstocks. Starch-containing feedstock must first be transformed to sugar or dextrin through an enzymatic process. Saccharification converts other complex sugars to simple sugars, which are then fermented to ethanol.

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