

Saccharomyces cerevisiae Bio-Ethanol Production as an Alternative Source of Sustainable Energy

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ABSTRACT

Current world energy demand is based on fossil fuels, which will vanish in coming decades. Renewable energy especially biofuels has attracted great interest as solutions to the current energy problem. Among available biofuel resources, bioethanol seems to be an efficient alternative thus, *Saccharomyces cerevisiae* a well-established organism for bioethanol production. However, during fermentation process, yeast cells experience various stress conditions and inhibitors hampering its efficacy for commercial bioethanol production. To overcome these yeast cells, adopt different signal transduction pathways. In this review, common and least explored carbon feedstock which can be readily converted into bioethanol are highlighted. The various protectants, genes, and pathways which can be tempered to engineer yeast strains are discussed. Thus, we have suggested strategies to utilize this lucrative alternative for sustainable bioethanol production.

Keywords- Biofuels, Fermentation, Feedstock and Yeast

I. INTRODUCTION

Human population has dramatically increased in the past decades, stretching the finite fossil fuels resources. Fossil fuels, coal, natural gas, oils account for 90% of total energy demand in the world. However, fossil fuels are limited and are major contributors of greenhouse gasses. Renewable energy is an alternative and among available resources, biofuels seem to be an efficient and sustainable energy. Current biofuels for bioethanol and biodiesel production are based on sugar crops. However, food versus fuel dilemma jeopardizes its long-term usage. Non-food crops (switchgrass, poplar, and willow), algae and Genetically Modified Organisms (GMOs) are other sources for biofuel production. Till date, yeast - *Saccharomyces cerevisiae* is considered to be ideal microorganism for ethanol fermentation (Lam *et al.*, 2014). It can utilize various feedstock for bioethanol production which are discussed below.

Bioethanol Production from Carbohydrates

Carbohydrates present in starch, cellulose, and hemicellulose are used for bioethanol production. Sugarcane juices, molasses, and corn are primary

feedstock used worldwide for bioethanol (Wilkie, 2000). Starch, a polysaccharide of glucose is obtained from corn, barley, wheat, rye, potato, sorghum, and cassava. For the production of bioethanol starch-containing feedstock must be first converted to sugar or dextrin by an enzymatic process and often enzyme used is amylase. Other complex sugars are converted to simple sugars by Saccharification process and are fermented to ethanol (Naik, 2010).

The biofuel produced from starch, sugars, animal fats and vegetable oils are referred as 'first generation biofuel'. Food and fuel dilemma however, jeopardizes its large-scale commercial production.

In coming decades, world population is expected to be about 9 billion and around 2.5 billion more people will be added by 2050 (Godfray *et al.*, 2010), thus, hampering sustainability of food crops for biofuel production. Moreover, insufficient supply of these crops hampers its long-term usage and its commercialization. As an alternative, lignocelluloses feedstock 'second generation biofuel', can be used (Kumar, 2009). Lignocellulose includes agricultural waste (straw of rice, wheat, corn, and sugarcane bagasse), nonfood plants like poplar, napiergrass, switches grass, paper waste, agro-industrial waste, water hyacinth and sawdust (Yasuda *et al.*, 2014). Being non-food crops it seems to be sustainable energy resource.

II. BIOETHANOL FROM AGRICULTURAL WASTE MATERIALS

Crops like corn, wheat, and sugarcane are primarily used for food. So, the sufficient production of these crops for fuel remains the major obstacle in the production of bioethanol (Cheng, 2011). The major agricultural wastes are straws of corn, wheat, and rice and sugarcane bagasse. These waste material don't have any nutritional value, easily available and are cheap. Moreover, it does not require separate agricultural land, water supply, fertilizers, and energy sources. Most of the agricultural waste materials are either left to rot in the field for composting or burnt in the fields. Rather than just disposing or burning these wastes it can be judiciously used as biomass for bioethanol production. Besides this feedstock like vegetable or fruit processing

wastes can also be used for bioethanol or biodiesel production.

Fermentation Stress Tolerance Mechanism

The yeast - *S. cerevisiae* is widely used in ethanol fermentation industry owing to its efficient conversion of sugars to ethanol (Fig. 1). However, during fermentation, it experiences numerous stress conditions. Stress conditions and an adaptive mechanism to overcome can be collectively called as 'Fermentation Stress Tolerance' (FST).

Tolerance to Ethanol

S. cerevisiae ferments sugar, starch, lignocellulose to ethanol but when the ethanol

accumulates above a threshold level, it inhibits growth, causes mitochondrial loss and eventually kills the yeast cells (Bai *et al.*, 2004; Ibeas and Jimenez, 1997). Increased ethanol level affects membrane stability, damages protein and destroys cell membrane. There are several studies which have shown the major pathways and genes involved in ethanol stress tolerance. The knockout strains developed by You *et al.*; showed tolerance to ethanol when supplemented with monounsaturated fatty acid (You KM, 2003). Inoue *et al.*(2000), demonstrated that strains lacking ergo sterol were sensitive to the moderate level of intracellular ethanol (Inoue *et al.*, 2000).

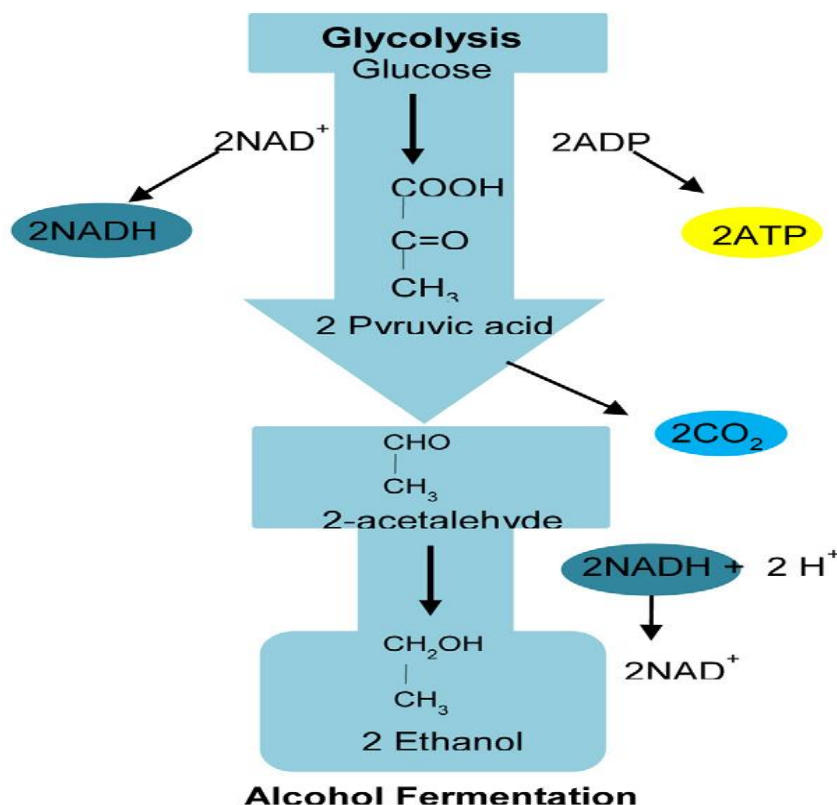


Figure 1: Ethanol fermentation (Inoue *et al.*, 2000).

Fig.1. Ethanol fermentation: One molecule of glucose (C₆H₁₂O₆) is converted into two molecules of pyruvic acid (C₃H₄O₃) during the process of glycolysis. Pyruvic acid is further decarboxylated to generate two molecules of acetaldehyde (CH₃CHO), which is reduced to ethanol (C₂H₅OH). During the process, there is a net gain of 2 molecules of ATP and one molecule of glucose is converted to two molecules of ethanol and two molecules of carbon dioxide (CO₂).

Yeast strains that over expressed genes like ARG4 and CAR1 responsible for the synthesis of arginine, showed to maintain the stability of cell wall and cell membrane (Cheng *et al.*, 2016). Our group has also shown that over expression of RPI1 increases ethanol tolerance by over 50 fold compared to WT. RPI1 over

expression strain is highly resistant to cell wall lytic enzyme Zymolyase (Puria *et al.*, 2009) suggesting; perhaps RPI1 improves the cell viability by strengthening the yeast cell wall.

A part from affecting plasma membrane, ethanol also denatures functional proteins and protein present in the cell membrane. In order to survive different environmental fluctuations, and to maintain the internal steady state homeostasis, cells have developed adaptive stress tolerance mechanisms. These cellular responses lead to change in gene expression and require signal transduction pathways to communicate from the sensors on the cell surface or cytoplasm to transcriptional machinery located in the nucleus to elicit a stress response, (Fig. 2) (Gasch and Werner-Washburne, 2002).

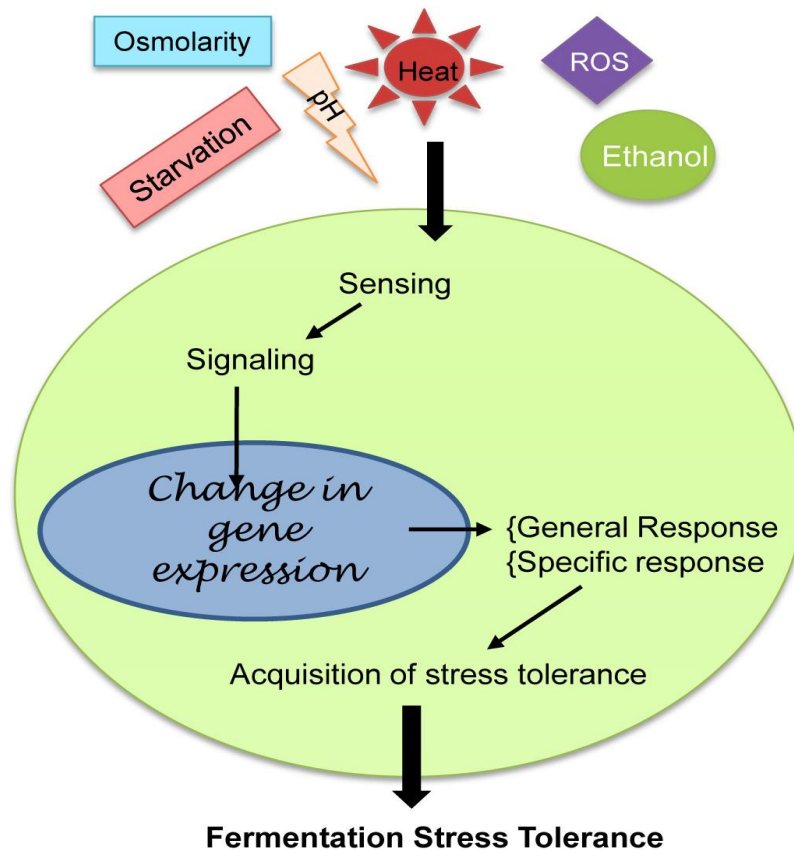


Figure 2: Fermentation Stress Tolerance Mechanism (Gasch and Werner-Washburne, 2002).

Fig. 2 Fermentation Stress Tolerance Mechanism: Yeast cells experience a plethora of stress conditions during fermentation namely high initial substrate concentration, nutrient deprivation, gradual accumulation of ethanol, the temperature rise of the fermentation medium, a decrease in pH, generation of reactive oxygen species (ROS). The cell sensing these signals by receptors over cell surface or intracellular receptors and transducer the signals for expression of certain genes under general or stress-responsive conditions. This expression of protective genes or detoxifier brings the adaptive stress responsive conditions called as fermentation stress tolerance.

Ethanol Toxicity in Yeast

The cell wall of *S. cerevisiae* is made of about 85% polysaccharides and 15% proteins. The main functions of the cell wall are to stabilize osmotic homeostasis, protect cells against physical damage, maintain cell shape and act as a scaffold for glycoproteins (Klis *et al.*, 2006). The main targets of ethanol stress are yeast plasma membrane and hydrophilic and hydrophobic proteins (Stanley *et al.*, 2010). On exposure of yeast to ethanol, the fluidity of cell membrane increases and the stability of membrane decrease (Mishra, 1989). Thus, ethanol affects the structure and function of the cell membrane. Ethanol also denatures various proteins present in the plasma membrane. Ethanol concentration of 2-6% inhibits endocytosis across the plasma membrane

(Lucero *et al.*, 2000). Ethanol breaks proton motive force, which pumps protons across the plasma membrane (Cartwright, 1986). Exposure of ethanol to yeast cells affects the activity of Pma1 membrane protein, an H-AT Pase, necessary to preserve intracellular pH and membrane potential.

III. GENETIC ENGINEERING TO IMPROVE YEAST STRAIN

C6 and C5 Carbon Substrate

Yeast can efficiently metabolize glucose and have less affinity for other carbon sources such as galactose. Moreover, in presence of glucose other metabolic genes are repressed by a process called as 'glucose repression' (Le Borgne, 2011). In this regard various genetically engineered strains of *S. cerevisiae* have been developed. Improved galactose up take and yield of ethanol were obtained when the genes encoding phosphoglucomutase and positive regulator of Gal4p were overexpressed (Ostergaard *et al.*, 2000). Overexpression of truncated TUP1 gene encoding repressor of transcription showed improved fermentation rates. Besides this lactose present in whey can be used to produce ethanol. But the commonly used *S. cerevisiae* for industrial ethanol production is not able to metabolize lactose.

For metabolizing lactose *Kluyveromyces fragilis* (Guimaraes *et al.*, 2008) or genetically engineered *S. cerevisiae* can be used. Starch rich materials are cheap and abundantly available; they can be used as feedstock for bioethanol productions. For fermentation of five carbon sources like xylose, yeasts with higher xylose fermentation rates like *Pichia stipitis* and *Pachysolen tannophilus* can be used (Jeffries, 1985; Jeffries *et al.*, 2007). Xylose can be converted to xylulose by using enzyme xylose isomerase, xylose reductase and xylitol dehydrogenase (Klimacek *et al.*, 2014). After conversion of xylose to xylulose, xylulose is phosphorylated to xylulose-5-phosphate and metabolized to ethanol. Klimacek *et al.* (2014) have developed an evolutionarily engineered strain of *S. cerevisiae* (IBB10B05) which can efficiently convert xylose to ethanol (Klimacek *et al.*, 2014). Konishi *et al.* (2015) developed a genetically modified strain of *S. cerevisiae* by using endogenous xylose digesting genes coding for sorbitol dehydrogenase, aldose reductase, and xylulose kinase to ferment xylose to ethanol (Konishi *et al.*, 2015). Besides xylose, arabinose is also not utilized by yeast, for economical ethanol production from lignocelluloses feedstock; it has to be also channelized for ethanol production. Wisselink *et al.* (2007) developed a genetically engineered strain of *S. cerevisiae* expressing *araA*, *araB* and *araD* genes from bacteria *Lactobacillus*

Saccharomyces cerevisiae Bio-ethanol Production, A Sustainable Energy Alternative S205 plantarum to ferment arabinose to ethanol anaerobically (Wisselink *et al.*, 2007).

IV. COMMERCIALIZATION AND FUTURE PROSPECTS

World energy consumption is increasing with the ever increasing population. Biofuels seem to be an efficient and sustainable energy resource. However, commercialization of biofuel is at infancy. The initial cost of investment, non-availability of arable land, seasonal nature of agricultural crops are some of the bottlenecks for commercialization. In this context algae considered to be the best option as it can thrive and profusely grow in non-arable land ranging from wasteland to aquatic ponds. Algal cell wall contents negligible lignin and intracellular stored starch granules can be readily converted to ethanol (Han *et al.*, 2015). For the economical production of ethanol from algal biomass, it is necessary that all the carbohydrate content of the algal feedstock is converted to ethanol. Simultaneous saccharification and fermentation (SSF), a single bioreactor is an alternative method for bioethanol production; it decreases fermentation costs by reducing equipment requirements. If countries worldwide need to be self-sufficient and reduce the crude oil import then research should be focused on identifying improved harvesting and oil extraction processes, increasing the biomass of biofuel

crops. All of these challenges can be resolved by genetic, molecular, and ultimately synthetic biology techniques (Lee, 2010).

V. CONCLUSION

Finally, looking into the near future of renewable energy and demand and supply, more options need to be explored as highlighted thus diversifying the sources.

Conflict of Interest

The authors contributed equally to the development of the manuscript and declare no conflict of interest.

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