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Saccharomyces Cerevsiae Bio-Ethanol Production, A Renewable and Sustainable Source of Energy

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ABSTRACT

Today, world energy demand is based on fossil fuels, which will vanish in coming decades. Renewable energy especially biofuels has attracted great interest as solution to the current energy problem. Among available biofuel resources, bioethanol seems to be an efficient alternative source of sustainable energy. Saccharomyces cerevisiae is a well-established organism for the production of bioethanol. However, yeast cells experiences various stress conditions during the process of fermentation. The efficiency of commercial bioethanol production is hampered by the inhibitors. To overcome these stress conditions, yeast cells adopt different signal transduction pathways. In this article, author discussed various protectants, genes and pathways which can be tempered to engineer yeast strains.

1. Introduction

The population of human being is increasing dramatically. This phenomenon is stretching the finite fuels resources. Fossil fuels, coal, natural gas, oil, account for 90% of total energy demand in the world. But, fossil fuels are non-renewable source of energy which is limited on earth and also are major contributors of green-house 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 (switch grass, poplar and willow), algae and genetically modified organisms and other sources for biofuel production. Till date, yeast, saccharomyces cerevisiae is considered to be ideal microorganisms for ethanol fermentation. It can utilize various feed stocks for bioethanol production which are discussed below.

2. Bioethanol Production from Carbohydrates

Carbohydrates are present in starch, cellulose and hemicellulose are used for the production of bioethanol. Sugarcane juices, molasses and corn are primary feedstock used worldwide for bioethanol. Starch is a polysaccharide of glucose and is obtained from corn, barley, wheat, rye, potato & orghum and cassava. For the production of bioethanol starch containing feedstock must be first converted to sugar or dextrin by an enzymatic process, after enzyme used in analyse. Other complex sugars are converted to simple sugars by saccharification process and are fermented to ethanol.

The biofuel production from starch, sugars, animal fats and vegetable oils are referred as “first generation biofuel.” Food vs fuel dilemma jeopardizes its large-scale commercial production. In coming decades, world population is expected to be 9 billion and around 2.5 billion more people will be added by 2050. 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, Lignocellulose feed stocks “second generation biofuel”, can be used. Lignocellulose includes agricultural waste (straw of rice, wheat and corn, and sugarcane bagasse), nonfood plants like poplar, napiergrass, switch grass, paper waste, agro-industries waste, water byacinth. Being non-food crops it seems to be sustainable energy resource.

3. 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 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 feed stocks like vegetable or fruit processing wastes can also be used for bioethanol or biodiesel production.

4. Fermentation Stress Tolerance Mechanism

The yeast, S. cerevisiae is widely used in ethanol fermentation industry owing to its efficient conversion of sugar to ethanol. 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).

5. Ethanol Fermentation

One molecule of glucose ($C_6H_{12}O_6$) is converted into two molecules of pyravic acid ($C_3H_4O_3$) during the process of glycolysis. Pyruvic acid is further is further decarboxylated to generate two molecules of acetaldehyde (CH_3CHO), which is reduced to ethanol (C_2H_2OH). 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 (Co_2).

6. 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. 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. Inoue et al; demonstrated that strains lacking ergosterol were sensitive to the moderate level of intracellular ethanol. Yeast strains that over expressed genes like ARG4 and CARI responsible for the synthesis of arginine, showed to maintain the stability of cell wall and cell membrane.

Apart from affecting plasma membrane, ethanol also denatures functional proteins and protein present in 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.

7. 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 transduce 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.

8. 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. The main targets of ethanol stress are yeast plasma membrane and hydrophilic and hydrophobic proteins. On exposure to yeast to ethanol the fluidity of membrane decrease. 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. Ethanol breaks proton motive force which pumps protons across the plasma membrane. Exposure of ethanol to yeast cells affects the activity of Pma 1 membrane protein, an H-AT Pase, necessary to preserve intracellular pH and membrane potential.

9. 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. Algae cell wall consist of negligible lignin and intracellular stored starch granules can be readily converted to ethanol. For the economical production of ethanol from algae biomass, it is necessary that all the carbohydrate content of the algae feed stock 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 technique.

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