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Response and tolerance of yeast to changing environmental stress during ethanol fermentation

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ABSTRACT

In development of yeast cell factories, the adequate stress tolerance of the yeast strains is the universal requirement for industrial fermentation. Primary stress factor is the end product toxicity which has to be accumulated to obtain economically feasible performance in industrial processes. Second stress factor is the concentration and composition of substrate used for ethanol production and the third stress factor is temperature gradients in the fermentor which influence fermentation rate and productivity. This review encloses the development in understanding the response of yeast towards various environmental stresses: heat shock, ethanol stress and osmotic stress. These stresses are fundamental challenges that are experienced by various microbes. Physiological changes such as the synthesis of compatible solutes and heat shock proteins also contribute to cellular survival. Different methods are available to improve the strain's survival under different stresses; thus, it will not only grow but also produce beneficial products in harsh environments. In combination with rational metabolic engineering to study and analyze processes of adaptive evolution, evolutionary engineering is a powerful strategy for improving industrially important, complex properties of yeast. Evolutionary engineering is an emerging field that draws on the most recent advances from a wide range of scientific and technical disciplines.

1. Introduction

Eukaryotic microorganisms are exposed to continuous changes in conditions that are prevalent in the natural environment, such as nutrient availability, hyper-osmotic and cytotoxic substances concentration. Eukaryotic cells encounter these stresses during their growth and reproductive cycles. Unicellular eukaryotes such as yeast possess a variety of mechanisms that enable them to recognize and respond to harsh environment conditions. The response can be obtained until harsh conditions do not occur at such a level that it became lethal for the yeast cell. The markers of stress response mechanisms are generally prevalent in the genomes, cytoplasm aand plasma membranes of the cells. During the process of fermentation, the yeast cells are dynamically exposed to a diverse and interrelated group of known stresses such as osmotic, oxidative, thermal, and ethanol stress and starvation. These stress conditions can considerably affect the yeast population and industrial fermentation efficiency [1]. As fermentation progresses, its efficiency and tolerance to relatively high levels of stresses are the traits that have made some yeast strains the keystone of modern alcoholic fermentation industries. There is a direct correlation between fermentation efficiency and stress resistance, which refers to the capability of a yeast strain to adapt efficiently to a changing environment and unfavourable growth conditions [2,3]. Industrial fermentation occasionally fails to go to completion or progresses at a slower rate; such problems are mainly due to stuck and sluggish fermentations, respectively. Several physiological factors can contribute to suboptimal fermentation such as increased ethanol concentrations, sugar stress and thermal stress [4,5].

Increased concerns about the depletion of available oil and climate change have resulted in a significant increase in oil prices, which has in turn resulted in increased demand for bioethanol production worldwide. Thus, efforts should now be directed towards identifying thermostable, ethanol-tolerant and stress-tolerant strains with the ability to utilize a broad spectrum of substrates (e.g. whey) and produce substantial amounts of the product [6]. Such strains of *Saccharomyces cerevisiae* are industrially important as they are prevalently used in baking, wine-making, brewing and the production of ethanol including fuel ethanol. Yeast cells have evolved to be exceptionally capable of surviving sudden and harsh changes in their external environment (such as temperature variation, changes in level of toxic substances). They must withstand fluctuations in temperature, osmolarity, toxic chemicals, the presence of radiations and long periods of nutrient

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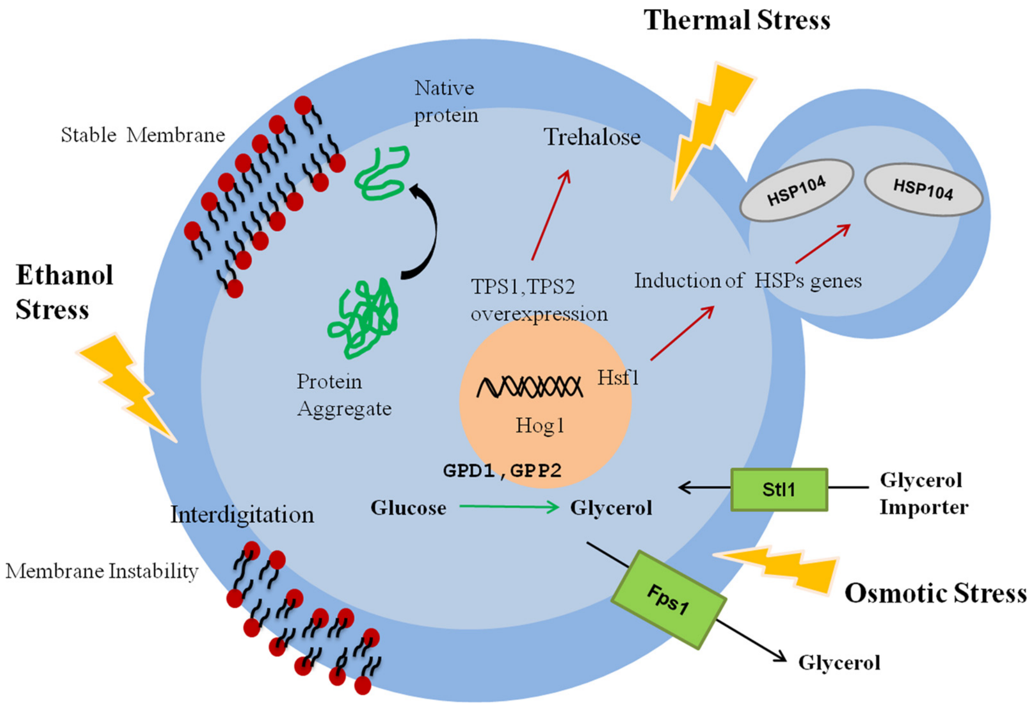

Fig. 1. Overview of the response of yeast to different stress. An osmosensing signal transduction pathway, called the high osmolarity glycerol (HOG) pathway, is encoded by gene Hog1. Hog1 mainly codes for the enzymes GPD1 and GPD2, which catalyze the conversion of dihydroxyacetonephosphate (DHAP) through glycerol-3-phosphate (G3P) to glycerol. Fps1, an osmosis-sensitive channel for glycerol diffusion, supports glycerol accumulation, which increases internal osmotic pressure. Glycerol is actively transported inside the cell by the H^+ symporter, encoded by Stl1. The enzymes required in trehalose biosynthesis behave as stress-responsive proteins: *TPS1* gene encoding trehalose-6-phosphate synthase (TPS) and *TPS2* gene encoding trehalose-6-phosphate phosphatase (TPP). Different heat shock proteins are induced by the heat shock transcription factor Hsf1.

starvation as well as of cell's desiccation. Growth under such diverse conditions requires maintenance of the internal system; however, the cellular programme essential for its maintenance varies depending upon the external challenges that the cell must face. Moreover, any unexpected change in environmental conditions causes the cell to adjust rapidly its internal environment in a way that is required for growth in the new conditions. The yeast cells use various mechanisms of cellular protection which are switched on when cells are exposed to stressful conditions. Furthermore, it has been described in literature that some yeast cells show a feature called as cross-protection against different stresses where cells exposed to a mild dose of one stress become resistant to large and usually lethal doses of other stresses [7]. This cross-stress resistance (i.e. resistance to a second, harsh stress after a different type of mild primary stress) is the consequence of activation of both a specific and general stress response programme, which is known as the environmental stress response (ESR). ESR upregulates the genes required to survive in stress conditions and provides defence from initiating stress. ESR plays a potential role in inducing mutagenesis in presence of stresses that can cause genetic instability in microorganisms. On the basis of such mutations, several researchers reported the phenomenon of 'adaptive evolution' or adaptive mutation [8]. However, the phenomenon underlying the ESR remains unexplored.

The main stress responses studied during the fermentation are as follows: response towards temperature, osmotic stress, nutrient starvation and the ethanol produced during the fermentation. This review summarizes the current understanding on the mechanism of stress tolerance in yeast cells during challenging fermentation conditions. The first part of the review deals with the different types of stresses along with their molecular mechanism encountered by the yeast cell during the fermentation, and the second section deals with the improvement in stress tolerance and ethanol production strategies. The conclusion highlights that the information presented in this review can lead to scientifically wide-ranging strategies for the improvement of fermented yeast strains.

2. General stress response in yeast

The response towards stress in yeast has evolved for protecting the cellular machinery and repairing the damage caused by the stress. Cells must respond to different physical stimuli such as nutrient concentration, osmotic stress and temperature changes. In eukaryotes, different groups of mitogen-activated protein kinases (MAPKs) play a critical role in intracellular signalling. MAPKs are also involved in the control of various stress reactions [9,10]. It appears that cells employ different MAPKs in various ways such that they organize the activities of various target proteins such as stress proteins or transcription factors. In *S. cerevisiae,* the high-osmolarity glycerol (HOG) pathway seems to specifically mediate responses to osmotic stress, while related pathways in fission yeast and other mammalian cells mediate protective responses to other stress conditions. The HOG pathway includes various transcriptional regulators of glycerol biosynthesis. Hog1 is a highly conserved protein that is required for stress adaptation and to modulate cellular metabolism [11]. Moreover, general stress-responsive genes are controlled by stress response elements (STREs) through the transcriptional factors Msn2p and Msn4p [12,13]. These factors bind to STREs (CCCCT) and mediate protein kinase A-dependent gene expression. The deletion of both *MSN2* and *MSN4* resulted in sensitivity to oxidative, thermal and osmotic stresses [14]. Changes in the structure and metabolism of an organism are caused by stress or environmental stimulus and act as expression activators for genes implicated in the synthesis of specific compounds that protect the organism. The environment around the yeast in distilleries is very different from the controlled laboratory environment. The substrate used in distilleries itself possesses some compounds at toxic levels (e.g. phenols, potassium, aluminium, cadmium and iron) and high sugar concentrations, which can cause osmotic stress [15,16]. Wheals and co-workers [17] reported that high sugar concentrations lead to high ethanol concentrations [8–11% (v/v)], which in turn trigger stress responses in yeast. Excess generation of reactive oxygen species (ROS) is the main consequences of heat stress, which results in oxidative stress in many species [18]. During stress response, heat shock response (HSR) is the most powerful adaptation mechanisms. It is a highly protective transcriptional programme that leads to changes in gene expression and results in the repression of the protein biosynthetic capacity and the induction of genes encoding the heat shock proteins (Hsps). Hsps function as molecular chaperones is to protect thermally damaged proteins from aggregation and unfold and refold damaged proteins as shown in Fig. 1. Ethanol and temperature tolerance are important and interactive features in industrial yeast