Abstract

Nitrosative stress is a hostile condition mediated by reactive nitrogen species (RNS). Macromolecules like DNA, proteins, lipids are very vulnerable to RNS. Protein modifications like protein tyrosine nitration (PTN) and *S*-nitrosylation are major markers of nitrosative stress. One of the best system to study nitrosative stress is *Saccharomyces cerevisiae*. Thousands of studies have been conducted by using *S. cerevisiae* to explore molecules involved in stress response, signal pathways, antioxidant system etc. but glucose metabolism and subsequent ethanol production under nitrosative stress was poorly understood. Here, metabolic response of *S. cerevisiae* has been characterized with special reference to the ethanol production under nitrosative stress mediated by acidified sodium nitrite (ac.$NaNO_2$) or *s*-nitrosoglutathione (GSNO).

Major findings of this study suggest no significant loss of cellular viability of *S. cerevisiae* in presence of 0.5 mM Ac. $NaNO_2$ or 0.25 mM GSNO as compared to the control where stress was not applied. These doses were riveted as the sub-toxic dose under experimental condition. In addition to it, growth rate of *S. cerevisiae* was found to be unaffected under these sub-toxic doses as compared to control. Redox homeostasis altered significantly, with a sharp increase in the specific activity of antioxidant enzymes i.e. glutathione reductase and catalase under sub-toxic dose of ac.$NaNO_2$ and GSNO. Confocal microscopy study revealed generation of RNS only in presence of stress inducing agents. Whereas presence of reactive oxygen species (ROS) was found in both control and treated samples without any significant differences.

Aconitase, which catalyzes the conversion of citrate to isocitrate in the tricarboxylic acid (TCA) cycle, is known to be affected under nitrosative stress. Under the specified experimental condition, it was found that enzymatic activity aconitase was strongly inhibited in the presence of 0.5 mM ac.$NaNO_2$ or 0.25 mM GSNO. Subsequent gene expression analysis also revealed that *ACO2* was affected whereas the expression level of *ACO1* was slightly higher in presence of 0.5 mM ac.$NaNO_2$. In addition to this, ethanol production increased by 1.3 fold and 1.5 fold respectively in presence of 0.5 mM ac.$NaNO_2$ or 0.25 mM GSNO as compared to the control. Volumetric productivity and yield of ethanol were also improved under the stress condition. Furthermore,

increase in alcohol dehydrogenase (ADH) enzyme activity was also observed under nitrosative stress. qPCR study revealed that gene expression of *ADH3* was significantly higher under the stress condition. Whereas western blot analysis with pure aconitase revealed that it was prone to both PTN and *S*-nitrosylation but pure ADH was not. Additionally, activity of some important enzymes of the TCA cycle, like citrate synthase, isocitrate dehydrogenase etc. were found to be negatively affected under stress. On the other hand, activity of enzymes related to malate metabolism and alcoholic fermentation were found to be increased under 0.5 mM ac.$NaNO_2$ mediated nitrosative stress. Altogether, a metabolic reprogramming towards fermentation was observed under nitrosative stress. Furthermore, ethanol production was optimized by using nitrosative stress exposed immobilized *S. cerevisiae* cells that were grown in a minimal medium containing molasses and ammonium sulfate. By performing CCRD-based RSM, optimized condition of ethanol production was determined. Overall, obtained data showed that maximum ethanol (35.24 g/L) production after 24 h of incubation. This is the first report of this kind where ethanol production by *S. cerevisiae* cells under nitrosative stress has been shown. This study has the potential to be significantly important in industrial ethanol production.