COMPARATIVE ANALYSIS OF GROWTH KINETICS, CONCENTRATION OF SUGAR AND ALCOHOL TOLERANCE OF VARIOUS YEAST STRAINS ISOLATED FROM FIVE DIFFERENT SOURCES

Sunil Kumar Verma*1, Ramsha Hussain*2

*1,2Department of Biotechnology, B N College of engineering, Lucknow, INDIA.

e-mail: Sunilkumarverma.6009@gmail.com.

ABSTRACT

Saccharomyces cerevisiae is used globally for bio-ethanol and alcoholic beverage production but its activity gets reduced due to the effect of accumulation of intracellular produced ethanol on cell vitality. Previously, a wide variety of yeast strains and their culture conditions have been used to reduce the impact of ethanol stress on gene expression but are influenced by various environmental factors. There are some similarities in gene ontology due to the consequences of ethanol assault that states that the stress response of Saccharomyces cerevisiae due to ethanol is compromised by the energy production constraints that ultimately leads to increased expression of genes associated with mitochondrial TCA cycle and glycolysis function and the decreased expression of ATP mediated growth associated processes. In the present investigation, Saccharomyces cerevisiae was taken as standard; distinct yeast strains was isolated from five different sources and their growth was observed under culture condition and ethanol estimation was done by DNS method. In alcohol tolerance test, yeast strain with best growth rate was utilized to tolerate ethanol stress. The molecular basis of the alcoholic tolerance of yeast strain can facilitate genetic engineering to develop strategies for improving its activity during ethanol stress.

Keywords: Sugarcane, Ethanol, Yeast, Growth, Alcohol tolerance, DNS test.

I. INTRODUCTION

Bio ethanol from Saccharomyces cerevisiae

Sake yeasts are known as Saccharomyces cerevisiae and are a good producer of ethanol with yield of approximately 20% (v/v) in sake mash (Watanabe et al. 2011). Fermentation rates of sake yeasts are higher and they are less tolerant to high concentrations of ethanol. So, there are a quite few challenges to prevent sake quality degradation caused by the death of yeast cells in the later stage of the Carbon dioxide (CO2) is the third major component and is present in 0.03% in atmosphere. Carbon dioxide emission from the burning of fossil fuels is considered to be a major source of global warming. Consequently, there is a need for alternative sources of energy particularly, carbon-neutral sources of energy (Ramos et al 2013). Bio ethanol, a renewable fuel, have the potential to replace nonrenewable sources and can be considered as an alternative to fossil fuel. Microbial production of ethanol is of greater significance because it can be obtained from renewable energy sources (Demirbas et al. 2008). A wide variety of ethanol producing plants (Sugarcane, Starch and lignocellulosic material) are being used to enhance the supply of ethanol and researchers are investigating new ways to improve the yield of ethanol production (Weijde et al. 2013).

In the United States, Corn is a major producer of ethanol (Schmer et al. 2014) while Brazil mainly utilizes sugarcane for ethanol production. Due to the consequences of international oil crisis, Brazilian bio ethanol industry has developed rapidly in the last few decades and sugarcane productivity has also enhanced due to genetic improvements in cultivars (Gnansounou et al. 2005). Technological and scientific advancement leads to new varieties of sugarcane fermentation process and agricultural management and engineering have increased the efficiency of Brazilian bio ethanol distilleries (Della-Bianca et al. 2014).

Fermentation of sake mash having high ethanol concentrations (Stanley et al. 2010) and hence a wide variety of ethanol-tolerant yeasts are bred and used in sake brewing. Particularly, sake with a mild taste along with little acidity, bitterness or astringency easily gets affected (Yamaoka et al. 2014). Selection of new yeast strains could be a promising way to enhance the production of fuel on a large scale (Kumar et
Industrial fermentation processes have various stressful conditions (e.g., ethanol concentration, ionic stress, osmotic pressure and temperature) on yeast that influence its activity and performance and also the kinetics during alcoholic fermentation (Bleoanca et al. 2013). Variation in temperature, pH and concentration of sugar are the most studied stress inducers in living cells (Arroyo-Lopez et al. 2009) but the alcoholic stress also has a great role on the growth kinetics and performance of yeasts (Da Silva et al. 2013) since ethanol is an intracellular product and its accumulation leads to the death of yeast cells (Nagodawithana et al. 1976). The present work proposes to study the growth kinetics of indigenous various strains isolated from different sources, ethanol estimation from these sources by DNS method and the alcohol tolerance of yeast strain having high growth rate in stressful condition of high ethanol concentration against standard Saccharomyces cerevisiae.

II. METHODOLOGY

Yeast Strains
The indigenous Yeast strains were obtained from different sources (Grapes, Orange, TAPI, Baker’s yeast and Sugarcane) and used as a strain to enter a quiescent state in the stationary phase for ethanol estimation by DNS method.

Media and culture conditions
The yeast strains were grown on YPD medium (1% yeast extract, 2% peptone, 2% dextrose). Solidified media contained 1.5% agar. A static pre-culture of yeast was grown at 30°C for 2 days in 25 ml of YPD medium. Fermentation process was carried out in 1-L Erlenmeyer flasks fitted with silicone stoppers containing 500 ml of YPD medium inoculated with approximately 10^6 cells/ml from pre-cultures at 30°C under static conditions without agitation. Cell growth was measured by the serial dilution of samples onto YPD agar plates. The colonies appeared were further purified using ampicillin antibiotic to prevent bacterial growth. Pure colonies were then isolated and kept in liquid YPD media. Cells obtained from the pre-culture were used for the analysis of metabolites.

Growth Kinetics
To analyze the growth of S. cerevisiae, yeast cells (1 x 10^6 cells/ml) obtained from different samples were inoculated into 250 ml YPD medium (1% w/v yeast extract, 2% w/v peptone, and 2% w/v dextrose) and grown overnight in an orbital shaker (150–180 rpm) at 30°C. Under this condition, Saccharomyces cerevisiae grows well. Cultured cells were centrifuged at 20,000 × g for 5 minutes at room temperature and washed and re-suspended in DNS. At stipulated time intervals (1, 2, 3, and 8 h), growth of cells was measured at 550 nm. Growth of respective S. Cerevisiae species was differentiated by measuring the specific growth rate (\( \mu \)). Where, is the specific growth rate, represented the number of cells at log phase, 0 represented initial number of cells, 2 was the time taken to reach stationary phase and 1 was the time when the cells entered the log phase. Specific growth rate values for different samples were differentiated from the exponential phase. During this phase, growth of cells per unit time is proportional to the initial cell concentration (Nordin et al. 2015).

Estimation of reducing sugar by 3, 5 - di-nitrosalicylic acid (DNS) method
0.5 ml of DNS reagent was added in 0.5 ml of culture supernatant from each sample and volume was made up to 9 ml with distilled water. This mixture was shaken well and boiled so that the color gets changed and incubated at 30°C overnight. The intensity of color change was measured at 600 nm at stipulated time intervals (1, 2, 3 and 8 h) for the estimation of reducing sugar during the growth of yeast cells.

Ethanol tolerance test
The yeast isolate obtained from sugarcane was tested for ethanol tolerance. The yeast strain was inoculated in 30 ml of YPD broth containing different concentration of ethanol (control, 5%, 10% and 15% w/v). The tubes were incubated at 30°C overnight. Viability of yeast cells were checked by measuring absorbance at 660 nm at stipulated time intervals (1, 2, 3 and 8 h).
III. RESULTS AND DISCUSSION

Growth Kinetics

A significant difference in the growth rate of yeasts isolated from different samples was observed after 2 h, indicating the exponential stage of yeast strains. Growth of yeasts results in turbidity which is an index of its growth. The suspended yeast cells in the culture, when interpreted, the passage of light, allowing less light to emit and reach the photoelectric cell. The amount of light energy transmitted through the suspension is measured as percentage of transmission from the Spectrophotometer at 0.1% to 100%. The density of cell suspension is expressed as optical density which is directly proportional to concentration of yeast cells. The optical density measured at 550 nm at different time intervals is shown in table I and the growth curve of yeast obtained from different samples was plotted and is shown in figure I.

![Growth Curve of Yeast Saccharomyces cerevisiae obtained from different sources.](image-url)
Table-1: Optical density measured at 550 nm during the growth of yeast from different sources

Estimation of sugar by 3, 5 - di - nitrosalicylic acid (DNS) method

DNS reagent is used for the estimation of sugar concentration in a particular sample and upon fermentation, yeast converts carbohydrate and sugars to carbon dioxide and alcohols. During the growth of yeast cells, DNS reagent interacts with the sugar present in sample and the change in color is observed. The intensity of color changed is expressed as absorbance or optical density which is directly proportional to the concentration of reducing sugar in the sample and is shown in table II and the graph from the observed data was plotted which is shown in figure II.

Fig-2: Sugar estimation curve during the growth of yeast obtained from (a) Grapes (b) Orange (c) TADI (d) Baker’s Yeast (e) Sugarcane and (f) Comparison chart of Sugar estimation of Yeast Saccharomyces cerevisiae obtained from different sources.
Table 2: Optical density measured at 600 nm for the estimation of reducing sugar during the growth of yeast from different samples.

Ethanol tolerance test

The yeast isolate obtained from sugarcane was tested for ethanol tolerance. The yeast strain was inoculated in 30 ml of YPD broth containing different concentration of ethanol (control, 5%, 10% and 15% w/v). The tubes were incubated at 30°C overnight. Viability of yeast cells were checked by measuring absorbance at 660 nm at stipulated time intervals (1, 2, 3 and 6 h) and are shown in table III and the graph from the observed data was plotted which is shown in figure III.

Table 3: Optical density measured at 660 nm from the yeast obtained from sugarcane at different concentration of ethanol.
**IV. CONCLUSION**

*S. cerevisiae* is one of the microorganisms which are used universally for the production of bio Ethanol (Sivasakthivelan et al. 2014). Efficient yeast strains with ethanol tolerance should be used to improve the yield of ethanol in the fermentation process that leads to the reduction of Distillation costs and also save the environment from harmful pollutants. Yeast was isolated from various sources such as Grapes, Orange, TADI, Baker's yeast and Sugarcane. The kinetic Behavior of the growth of yeast was studied and the pattern of growth curve showed a sigmoidal Curve. The growth curve pattern showed that yeast obtained from Sugarcane had best growth Curve and the slope of sugar estimation curve of Sugarcane is continuously decreasing as Compared to all other samples taken and the yield of ethanol production increases with decrease In sugar concentration (Tahir et al. 2010). Yeast sample with best growth curve among all the Samples were taken to alcohol tolerance test with 5%, 10% and 15% alcohol concentration in which sugarcane sample showed best alcohol tolerance.

**V. REFERENCES**


