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To Reduce the Potential Environmental Stresses on Saccharomyces Cerevisiae during Alcohol Fermentation from Sugar Cane Molasses

KEYWORDS: Saccharomyces Cerevisiae, UV mutation, Transformation methodes , Decreasing use of water, Increasing ethanol production

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ABSTRACT *In India, the Past majority of ethanol is produced from sugarcane molasses, a by-product of sugar. In the future it may also be produced directly from sugarcane juice. The main objective of this study is to develop an economic framework to determine the implications of the 2017 blend mandate for India's food and energy security and allocation of land and water between food and fuel production. In the present work, studies were carried out on the ethanol production by Saccharomyces cerevisiae under stationary culture. Cane molasses in different concentration was used as sugar source for maximum conversion of reducing sugar into ethanol. The substrate was optimized after maintaining different levels of sugar concentrations (15-30%), medium pH (4.0-5.5), incubation temperatures (25-40°C), volume of fermentation medium (200-350 ml) and yeast cells gave significant results up to four consecutive batches. Rate of ethanol production was maximal with the free cells. From studies with PEGz strain, it is revealed that the strain is as efficient as the IIS strain, But due to its transformation and mutation, the strain showing resistance to high sugar conc and have much temperature tolerance, than IIS particularly much efficient in ethanol production.*

INTRODUCTION

Considering the importance of the distillery industry in the national, regional and the local economy, a number of researches have been worked on the various aspects of ethanol production improvement. taking them into the account ,an attempt has been made to review the available literature in this field.

Ethanol production is among the oldest technology and is produced commercially by fermentation of cereal grains, molasses or other materials with high starch and/or sugar contents. The fermentation process involves conversion of sugars to alcohol and carbon dioxide by the yeast Saccharomyces cerevisiae.

The principal biological agents of fermentation are yeasts belonging to the genus Saccharomyces, which can catalyse alcoholic fermentation to ethanol production. In recent years, yeast strains of Saccharomyces cerevisiae were extensively studied for biotechnological properties enhanced. Industrial yeast strains have to resist to the stress conditions rapidly and to adapt easy by modifying their metabolic activities to avoid substantial viability loss.

The great majority of ethanol produced in the world is from sugarcane molasses. The objective of this study is to review the state of the art in industrial bioethanol production from a modern biotechnological methods point of view for productivity improvement.

1) Kiransree et al. (2000) produced with molasses containing 14% sugar, maximum 53.2 and 45 (g/l) at 30 and 40°C respectively by thermo-tolerant strain. That strain showed 12% W/V ethanol tolerance. Isolated strain was also characterised for its ethanol producing ability using various starchy substrates in solid state and submerged fermentation.

2) Some successful attempts to adapt yeasts to high temperatures have been described. Saccharomyces cerevisiae yeasts, capable of fermenting at 40 and 45°C, have been obtained using progressive cultures (Rikhvanov et al., 2001). Thermotolerant yeast strais have been obtained by selecting survivors after a shock process at relatively high temperatures. Some authors demonstrated that during 15 min of incubation at 55°C resulted tolerant cells to higher temperature than nonincubated controls.

3) The term "transformation" was first used by Griffith to describe heritable changes in certain characteristics of Pneumococcus and is now used to describe DNA uptake in both prokaryotes and eukaryotes, as detected by consequent changes in phenotype. In his extensive and elegant study, Griffith showed that a non-reverting avirulent form of Pneumococcus that lacked a polysaccharide capsule could be "transformed" to a virulent form by injecting heat-killed encapsulated virulent bacteria along with the non-virulent inoculums into the peritoneal cavity of a mouse. He suggested that the "transforming principle" might be a component of the polysaccharide capsule.

4) More recently, Thompson et al. (115) reported that yeast cells pretreated in a LiAc/DTT/TE buffer, washed and then electroporated in 1.0 M sorbitol, gave transformation efficiencies of 0.5-1.4*10^{6}transformants/mg plasmid DNA. This pretreatment increased the yield of transformants 15- to 300- fold for strains that were refractory to conventional Electroporation.

Materials and methods

Microorganism and its maintenance:

The strain of Saccharomyces cerevisiae used in this work was isolated from an ethanolic fermentation of sugar cane molasses of manjara sugar-ethanol industry . S. cerevisiae was maintained at 4 oC on agar slants. The composition of the agar was (g l-1): yeast extract 3gm, peptone 5gm, glucose 10gm and agar 20gm, D/W 1L. The cultures were maintained by sub-culturing every 20-days and the test tubes were then incubated at 30 °C for 36 hr. The Baker's yeast, largely used in industrial process, was also used in this work. Commercial Baker's yeast was bought from German bakery, pune, Maharashtra.

Strain and Media

Yeast strains were grown on YEPD and utilized for fermentation in the molasses media with urea, DAP, sulphuric acid, distilled water and fermented at temperature 35oc, for 48

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hours.

Figure 1: Pre fermentation (inoculum)

Mutagenesis and mutant isolation

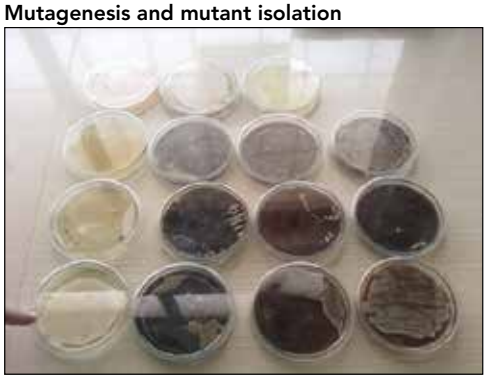

Figure 2: Isolated strains of Bakers yeast on concentrated molasses medium after UV mutation.

For the analysis of survival rates by UV mutagenesis, cells grown by shaking at 100 rpm for 18 h at room temperature on a YEPD medium were collected and suspended in sterile water. After the cell concentration was determined by counting, cells were spread on YEPD medium plates.

The two of which having 12 brix and 22 brix glucose concentration. The plates were placed under a UV lamp (Sylvania G30W) at a distance of 55 cm and were irradiated for various periods of time. Following irradiation, the plates were kept in the dark for 1 h and then were spread onto YEPD plates before incubating at 25°C for 3 day, the number of colonies were counted to determine survival rates .

Ethanol Fermentation

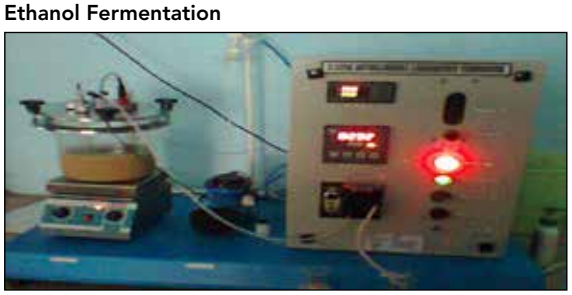

Figure 2:fermentation is carried out in lab fermtner

To evaluate substrate tolerance ability of the mutants, ethanol fermentation was carried out at room temperature using glucose as substrate at initial concentration of 15, 20, 25 and 30% (w/v). Then temperature tolerance of the yeast strains was monitored at 25, 30, 35, 40 and 45°C by using glucose concentration at 20% (w/v) . Furthermore, ethanol tolerance ability of yeasts was carried out by varying initial ethanol concentration at 1, 3, 5 and 7%. Samples from each experiment were taken at time intervals, centrifuged and supernatant was examined for viable yeast cells, reducing sugar concentration and ethanol concentration.

Analytical methods:
To determine the 0Brixof the sample weigh 100 gm of the given molasses, add 900gms of the cold water. Mix well till a homogenious mixture results.

The prepared solution is poured into a jar provided with overflowing through, until it overflows. The jar should be sufficiently wide so as to permit free movement of the brix hydrometer. This should free from air, suspended matters and insoluble impurities.the spindle is carefully lowerd into the solution should not be moistered. Care should be taken that the instrument floats freely and does not touch the bottom of cylinder or even side walls take readings.

To determine the Reducing sugar in the molasses use eynon and lane method.

To determine the volatile acidity of the fermented broth collect the distillate transfer it to the 250 ml conical flask, and titrate 0.1 NaOH upto the appearance of colourless to pink colour. note burrete reading.

Experimental Methods:
Yeast strains were isolated from the factory area and from bakery as control samples by the simple agar plate method on the YEPD media.

The yeast is confirmed by the morphological characteristics. and then this is used for further modification. Yeast DNA is isolated in the bulk quantity by common method and then separated by electrophoresis.Mutation is done by using UV light and transformation is performed by the Electroporation, Polyethylene glycol method, and Glass beads method. The strains are then optimized for the pH, temperature, sugar concentration and results were checked by fermentation of that strain.

Distillation is done by fractional distillation method. the filtrate was boiled at 780c and condenced.

Quantification of the residual sugars in the molasses
Molasses diluted is titrated against fehlings A and fehlings B in boiling conditions and note down the burrete reading. Reducing sugar% = 5.128/B.R.

Quantification of the ethanol produced by specific gravity:
Specific Gravity: W3 – W1 / W2 – W1
Then determine the % of ethyl alcohol corresponding to the apparent specific gravity at specific temperature.

Results & Discussions :
Result of the control :
The four strains namely Baker's yeast (present in the market as common yeast), Rapid performer 22(UV mutated and acclimatized to 22 brix sugar concentration), Rapid performer 12 (UV mutated and acclimatized to 12 brix sugar concentration), and industry isolated yeast (collected from the empty fermentation tanks, suspected areas) are used to further transformation and optimization practices.

After the isolation strains are traced for their original fermentation performance known as the control readings.

For the fermentation urea concentration was 4grams in 500ml, then fermentation had been done for 48 hours, later on distillation is done and ethanol is estimated by titration with 0.1 N NaOH, and phenolphthalein as indicator. The final RS tells about the sugar consumed.

Table 1: Result of the control UV mutated strains

Strain	pH	Temp (°C)	Sp.Gravity	I.R.S. (%)	V.A.	Alcohol %	F.R.S.
B.Y.	4.5	35	o.989	10.25	2673.84	7.78	0.854
Rp 22	4.5	35	0.986	10.25	3033.78	10.11	0.915
Rp 12	4.5	35	0.988	10.25	4621.78	14.92	0.416
IIS	4.5	35	0.980	10.25	2108.22	8.55	0.788

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Table2:Result of Transformed yeast strains ethanol recoveries

Strain	Alcohol %	Reducing sugar
ELx (B.Y.+Rp12)	5.49 (0.992)	0.827 (6.2 ml)
Ely (B.Y+Rp22)	8.50 (0.988)	0.453 (11.3 ml)
ELz (B.Y.+ IIS)	4.77 (0.993)	0.366 (14 ml)
PEGx (B.Y.+Rp12)	10.82 (0.985)	0.434 (11.8 ml)
PEGy (B.Y.+ Rp22)	9.27 (0.987)	0.557 (9.2 ml)
PEGz (Rp22+IIS)	12.38 (0.983)	0.416 (12.3 ml)
GLx (B.Y.+Rp12)	2.69 (0.996)	1.282 (4 ml)
G y (B.Y.+Rp22)	9.33 (0.987)	0.712 (7.2 ml)
GLz (Rp+IIS)	15.75 (0.979)	0.899 (5.7ml)

From the above transformation practices followed and results obtained we can conclude that the strain PEGz (Rp22+IIS) is the most performance oriented strain.

So we can go for the further optimization of this performer to get better ethanol yield with efficient sugar consumption.

Results of PEGz (Rp22+IIS) strain optimization

- At pH 4 the PEGz strain given 16% ethanol recovery.
- At temperature 30°C the PEGz strain given maximum 20% ethanol production & at 40°C given 12% production of ethanol.
- At 300gm/L sugar concentration PEGz strain given maximum 14% ethanol production.

11. Conclusion:

From the result and discussion it can be concluded that strain PEGz is the most performance oriented within the strains we have been taken for transformation.

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