Studies on Development of High Yielding Ethanol and Temperature Resistance Strain of Saccharomyces cerevisiae

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Abstract

Saccharomyces cerevisiae AB, an ethanol producing strain (3.0 %) was treated with 10%, 15% and 20% ethanol for 30, 60,120 and 180 minute. Only 5.5% colonies isolated by 30 minute. Incubation in 10% ethanol gave higher alcohol production (5.4%) than the parent strain. This strain when incubated in 15% ethanol for 60 minute, then only 4.5% alcohol resistant strains gave higher alcohol production (6.3%). The parent strain on farther exposure to 20% ethanol, there was decreases in alcohol production. Saccharomyces cerevisiae AB 810 an ethanol production strain (6.3%) was incubated at 35 ^{0}C, 40 ^{0}C and 45 ^{0}c temperature for 6, 12, 18 and 24 hours. Only 18% colonies isolated by 24 hour incubation at 35^{0}C temperature which gave highest ethanol production (6.5%). On further exposure at 45 ^{0}C temperature, all the resistant strains were killed, so there was no alcohol production.

Key words: *Saccharomyces cerevisiae, alcohol resistant and temperature resistant, ethanol production.*

I. INTRODUCTION

Alcohol fermentation is the most important example of microbial production of alternative energy. However, the yeast strains normally used in industrial process show a limited tolerance to ethanol, temperature and high osmotic pressure of the medium [1]. An important research field in alcohol fermentation is the search for new yeast strains with higher tolerance to temperature and ethanol ability to produce elevated concentration of ethanol. The isolation and selection of thermo and ethanol tolerant yeast strains [2, 3, 4, 5] and of strain capable of growing and fermenting media containing high percentages of sucrose have been reported [6]. The major areas of research interest in ethanol fermentation have been: i) increases ethanol concentration and specific ethanol production rate, ii) improvement of ethanol tolerance of yeast [7, 8, 9] and iii) development of continuous of ethanol fermentation process using high density cell culture [10, 11, 12]. In India, due to the scarcity of petroleum crude and the rate of depletion of fluid fossil fuel resources, it is necessary to produce more and more ethanol from agricultural carbohydrate products and biomass to satisfy the fuel combustion demand and other chemical industry demanded.

The present paper deals with the development of high yielding ethanol and temperature resistant strain of Saccharomyces cerevisiae to increases the rate of production of ethanol from carbohydrate materials and biomass.

II. MATERIAL AND METHODS

Microorganisms

The yeast Saccharomyces cerevisiae AB used in study was isolated from instant yeast supplied by Khotari Fermentation and Biochem Limited Company, New Delhi (India). This strain was used for the development of high alcohol resistant strains using 10%, 15% and 20% ethanol. The time of incubation of yeast concentration was 30, 60, 120 and 180 minutes. In the course of development of ethanol resistant strain by using above mention ethanol concentration, 1200 resistant strains were isolated. Out of this strain resistant strains only Saccharomyces cerevisiae AB860 which produced 6.5% alcohol (v/w %) more than mother strains of yeast 3% alcohol (v/w %) was selected for further studies.

Medium and culture conditions

The parent culture Saccharomyces cerevisiae was maintain from YPD (Yeast Peptone Dextrose) agar medium (1% yeast extract, 0.2% peptone, 2% dextrose and 4% agar, pH was adjusted to 4.5) slant

at 4 ^{0}C. The medium used for the formative production of ethanol contained glucose 5%, KH_2PO_4 0.1%, $NaNO_3$ 0.2%, $MgSO_4.7\ H_2O$ 0.05%, yeast extract 1% and pH 4.5. Glucose was sterilise separately and then added to the medium before incubation. The yeast cells were harvested by washing the slant with sterilise distil water and filtering the resulting cell suspended by absorption cotton. The cell density was adjusted to 2.6 x10^7 cells per ml of the suspension. The cell suspension was used for the inoculation of fermentation medium. Surface culture fermentation was carried out using 500 ml conical flask, each containing 150 ml of medium. The flask was then incubated at 28 ^{0}C for 48 hours.

Temperature resistant strain of Saccharomyces cerevisiae

The cell suspension of ethanol resistant strain cell of Saccharomyces cerevisiae AB860, containing 2.6 x10^7 cells per ml were then treated at 35 ^{0}C, 40 ^{0}C and 45 ^{0}C for 6, 12, 18 and 24 hours. After incubation for 6, 12, 18 and 24 hours the cell suspension in each case was diluted and plated out on YPD agar medium. The 780 isolated strains were selected for different stages of treatment with 35 ^{0}C, 40 ^{0}C and 45 ^{0}C temperature for ethanol production.

Determination of ethanol concentration

After alcohol fermentation the ethanol produce was determine by gas chromatography (GC) [Pye Unicam series ion] with flame ionising detector [FID] on a column of porapak-O using N_2 as carrier gas. The column temperature and detector temperature was 190 ^{0}C and 230 ^{0}C respectively. In each case 5 µL sample was injected. The quantitative calculation of ethanol concentration was made by measuring the peak areas of sample in calibration relative to the internal standard n-Propanol used as internal standard. Fermentable and non-fermentable glucose was measure by AOAC method [13].

III. RESULTS AND DISCUSSION

Development of ethanol resistant strain of Saccharomyces cerevisiae

A total of 860 isolates of yeast were selected after injection of 10%, 15% and 20% ethanol for varied time of incubation. Fig-1 shows that at 10%, 15% and 20% ethanol concentration, with the increases of time of incubation from 30 minute to 180 minute the percentage of survivor decreases from 92% to 4 %. It also suggests that the productive variant also decreases from 5.0% to 1.5 % at 10% ethanol concentration. Fig-1 also shows that during this experiment the strain Saccharomyces cerevisiae AB510 was isolated among the 560 strains which was 10% ethanol resistant and produce higher ethanol yield (5.4 %) over the parent strain (3%). During 15% ethanol resistant strain studies (Fig-1), 58% of the strains were killed at 60 minute incubation. Out of 200 isolates strains only 30% productive variants of 15% ethanol resistant strains Saccharomyces cerevisiae AB 810 obtain during 60 minute incubation produce (6.3%)

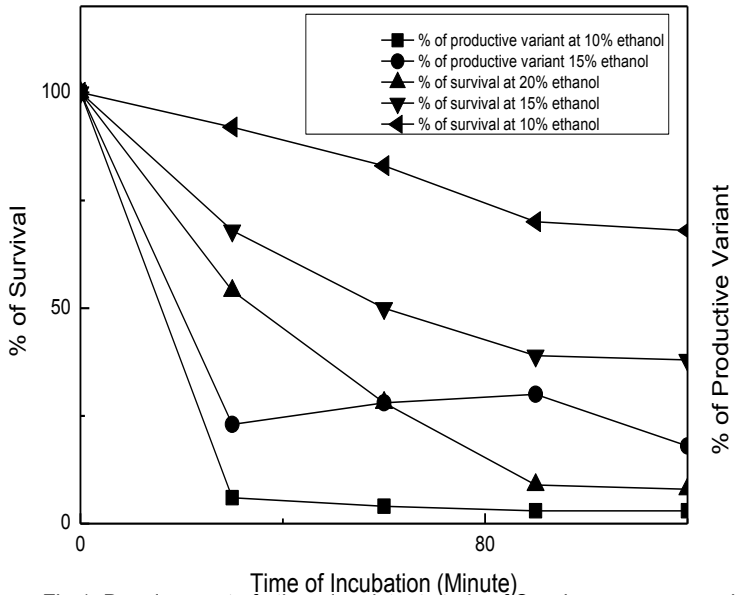

Fig 1: Devolopment of ethanol resistant strain of Saccharomyces cerevisiae

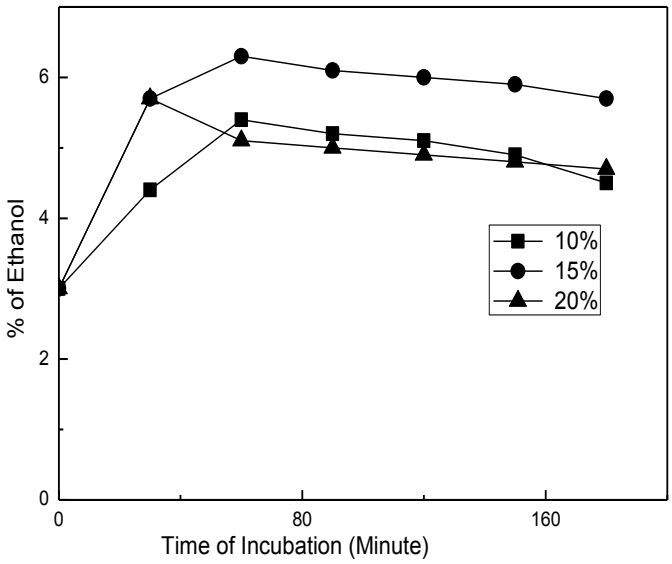

Fig 1a :Devolopment of ethanol resistant strain of Saccharomyces cerevisiae

ethanol. During 20% ethanol resistant strain studies, lethal effect was maximum at 180 minute incubation. No productive variants were isolated during this study. The 100 isolates were selected from different stages of treatment with 20% ethanol for ethanol production. It was observed from Fig-1a that the strain isolated from 20% ethanol incubated for 30, 60, 120 and 180 minute produced lower yield of ethanol than the 10% and 15 % ethanol resistant strains.

Development of temperature resistant strain of Saccharomyces cerevisiae

In the course of development of temperature resistant strain by maintaining Saccharomyces cerevisiae AB 810 at 35^{o}C, 40 ^{0}C and 45 ^{0}C for varied time of incubation like 6, 12, 18 and 24 hours. The 772 resistant strains were isolated. Fig-2 suggests that the percentages of survival of Saccharomyces cerevisiae at 35^{o}C and 40 ^{0}C decrease with the increases of time of incubation. It also suggests that during 45 ^{0}C the temperature resistant strains studies, after 18 and 24 hours incubation all the isolation strains were died. So there was no question of ethanol production which was supported by Fig-2a. This might be due to the inactivation of enzymes at higher temperatures after 24 hour incubation. No productive variants were isolated during 6 and 12 hour of incubation. It is evident from Fig-2 that at 40 ^{0}C temperature resistant strains studies, all the strains isolated after varied time of incubation were negative variants. But at 35 ^{0}C temperatures Fig-2 shows that 18% of productive variants isolated after 24 hour of incubation at high temperature produced highest yield of ethanol (6.3%). In our above studies we have isolating ethanol (15%, 60 minute) and temperature (35 ^{0}C, 24 hour) resistant strains Saccharomyces cerevisiae AB 810 X_1 which produce highest ethanol yield (6.5%).

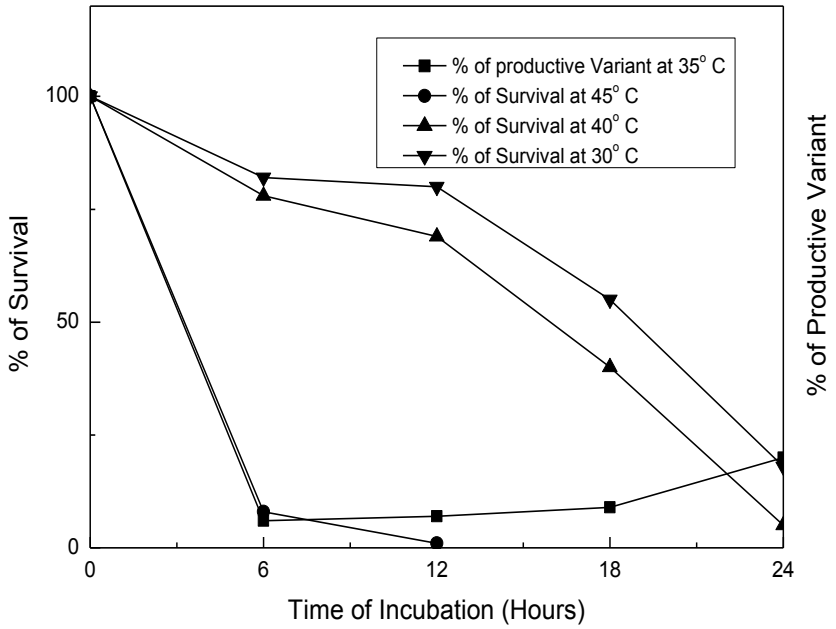

Fig 2 : Development of temperature resistant strain of Saccharomyces cerevisiae

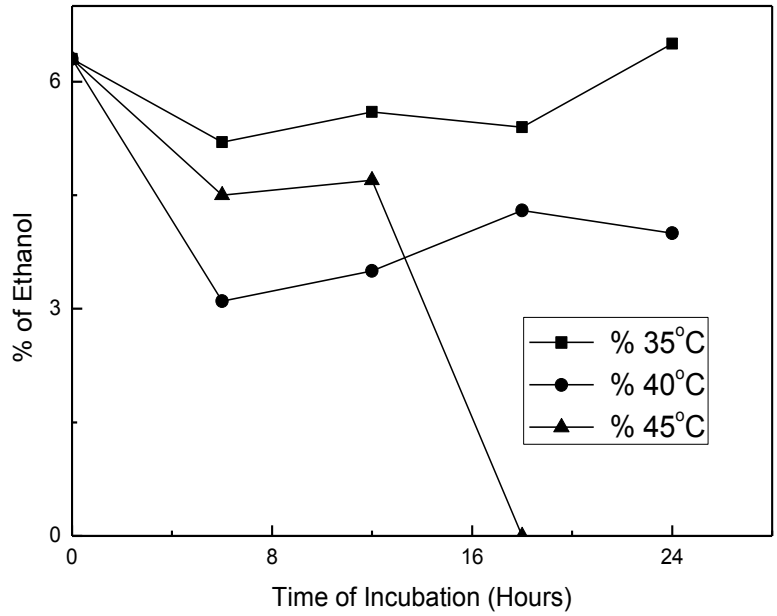

Fig. 2a : Devolopment of temperature resistant strain of Saccharomyces cerevisiae

IV. CONCLUSION

In the present studies, we have observed superiority of 10% and 15% ethanol resistant strains of higher alcohol production than the 20% ethanol resistant strains although the lethal effects are lower. Further studies are in progress to test this culture for its industrial utilisation for large scale alcohol production from agricultural carbohydrate and biomass.

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