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Production of Ethyl alcohol by Dual fermentation using both *Brevibacillus borostelensis* R1 and its partially purified α-amylase

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ABSTRACT

The basic objective is to study the effect of Brevibacillus borstelensis R1 and its partially purified α-amylase along with Saccharomyces cerevisiae in ethyl alcohol dual fermentation. The production of ethyl alcohol was estimated and analyzed for 30 days at regular intervals. Yeast extract, peptone and starch medium (YPS) was inoculated with Saccharomyces cerevisiae as control and with Saccharomyces cerevisiae and Brevibacillus borstelensis R1 as test. In our studies of dual fermentation, 5% co-strains of Saccharomyce cerevisiae and Brevibacillus borstelensis R1 showed maximum (3.37±0.23U/ml) alcohol production on the 21st day.

Keywords: Brevibacillus borostelensis R1, α-amylase, Saccharomyces cerevisiae, Dual fermentation, alcohol.

INTRODUCTION

Nielsen & Borchert[1] used α-amylases for starch hydrolysis in the starch industry. In starch liquefaction process starch converts into fructose and glucose syrups. Process requires α-amylase which is more active at high temperature. Gupta *et al.*[2] and Prakash & Jaiswal[3] reported the involvement of α-amylases in enzymatic conversion of starch (includes gelatinization, liquefaction and saccharification) in to maltose and glucose. Bloom *et al.*[4], Takasaki *et al.*[5], Bajpi & Bajpi[6], Shetty & Crab[7] and Akiba *et al.*[8], reported that many industries use α-amylases for the production of simple sugar glucose. The enzymes hydrolyze α -1, 4 glycoside linkages in the starch polymer in a random manner to yield maltose and glucose. Therefore alpha-amylase is extensively used in industries for the production of glucose.

For ethyl alcohol production, starch is the most used substrate due to its low price and easily available raw material in most regions of the world[9]. In beer industries microbial amylases are used to aid cereal amylases in the production of fermentable sugar[10]. The bioconversion of starch into ethanol involves liquefaction and saccharification, where starch is converted into sugar by using an amylolytic microorganism or enzymes such as α-amylase, subsequently by fermentation, sugar is converted into ethanol using an ethanol forming microorganism such as yeast *Saccharomyces cerevisiae*[11]. Among bacteria, α-amylase obtained from thermoresistant bacteria, *Bacillus licheniformis,* engineered strains of *Escherichia coli* and *Bacillus subtilis* were used during the first step of hydrolysis of starch suspensions[12]. Still further studies are needed to reduce the cost by dual fermentation method for ethanol production.

Ethanol formed by the bioconversion of starchy materials is commonly known as biofuel. In the present scenario, however, production cost is very high. Eventhough methods are developed to minimize cost by fermentation of starch to ethanol in one step using two different co-culture strains and low temperature cooking fermentation systems that succeeded in reducing energy consumption by approximately 50%, it is still necessary to add large amounts of amylolytic enzymes to hydrolyze the starchy materials to glucose[13&14].

MATERIALS AND METHODS

Estimation of amylase by DNS method

Maltose produced by the hydrolytic activity of α-amylase on α-1, 4 linkages present in polysaccharides, reduce 3, 5 dinitro salicylate to an orange red colored 5-nitro 3-amino salicylate which can be measured at 520nm. The starch substrate [0.5ml of 0.5% in 0.1M phosphate buffer (pH 6.8)] was mixed with 1% (0.2ml) NaCl in a test tube and pre incubated at 37^0C for 10 minutes. The supernatant collected from the centrifugation of the production media was used as enzyme source, 0.5ml of this was added to the reaction mixture. The reaction was terminated by the addition of 1.0 ml of 3, 5-dinitrosalicylic acid reagent [1.0 gm DNS in 0.8% NaOH, 60% Na K tartrate] after incubation at 37^0C for 15 minutes. The contents were mixed well and kept in boiling water bath for 10 minutes. Then they were cooled and diluted with 10 ml of distilled H_2O. The color developed was read at 520nm. One unit of enzyme activity is defined as the amount of enzyme that releases 1.0 mmol of reducing sugar (maltose) per minute under the assay conditions [15].

Ethyl alcohol dual fermentation

Yeast extract peptone starch medium (Modified medium of Yeast extract peptone dextrose) in gm/l: yeast extract 10, peptone 20, starch 20 at pH 6.0. Hundred ml of YPS medium was taken in two erlenmeyer flasks labeled as control and test. The erlenmeyer flasks were inoculated with 5% *Saccharomyces cerevisiae* as control, and test with 5% *Brevibacillus borostelensis* R1 & 5% *Saccharomyces cerevisiae*. Ethyl alcohol and maltose were estimated for 30days at an interval of three days.

Determination of Ethyl alcohol

The production of ethyl alcohol in control and test were measured by Potassium dichromate method [16].

Statistical analysis

All the experiments were conducted in triplicate. The results were given as mean value ± standard deviation. The conditions were analysed to determine the singnificant difference between the variables by one way ANOVA, two way ANOVA and correlation analysis by using the scientific graph pad (Prism 6.1version software). Analysis of variance (ANOVA) refers to the examination of differences among the sample means. It is used to examine the significance of the difference amongst more than two sample means at the same time.

RESULTS AND DISCUSSION

Ethyl alcohol dual fermentation

The application of α-amylase for ethyl alcohol production in Yeast extract, peptone and starch (YPS) medium is shown in fig. 1a. The production of ethyl alcohol was estimated by using standard curve as shown in fig.1b. The ethyl alcohol was analyzed for 30 days at regular intervals as shown in fig. 1c. YPS medium was inoculated with *Saccharomyces cerevisiae* as control and with *Saccharomyces cerevisiae* and *Brevibacillus borstelensis* as test. In our studies of dual fermentation, 5% co-strains of *S.cerevisiae* and *Brevibacillus borstelensis* R1 (test) showed maximum (3.37±0.23U/ml) alcohol production on 21st day.

Fig. 1:
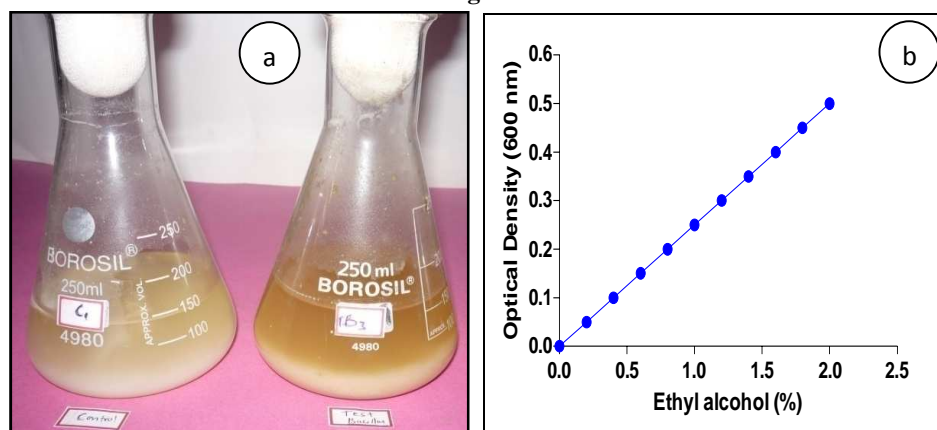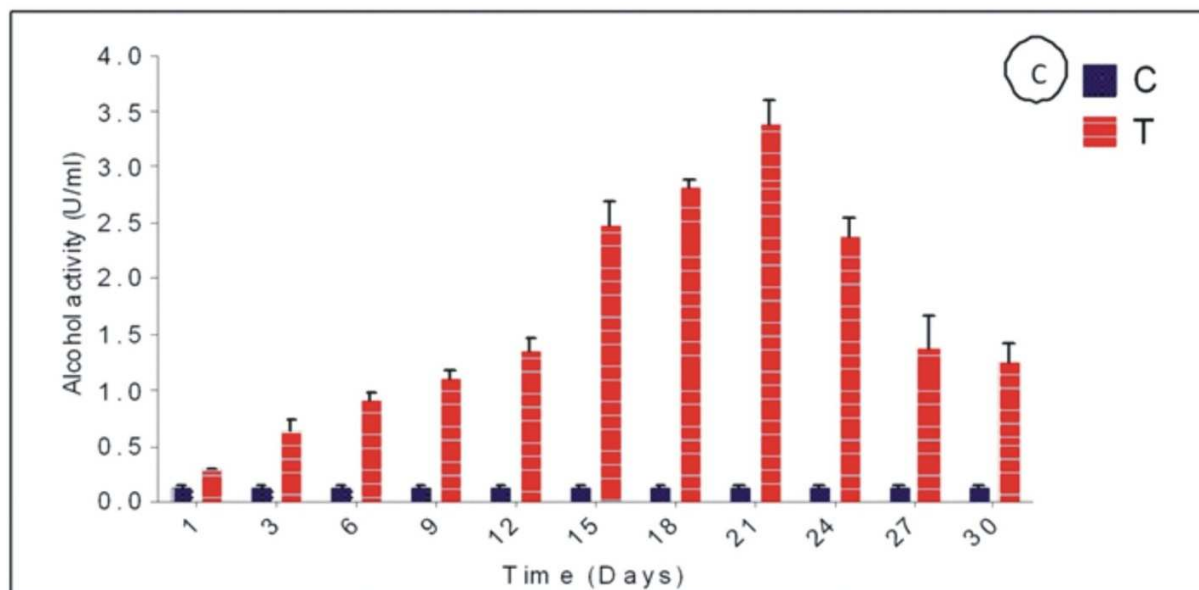
Y bars indicate the standard deviation of mean value.

**** P < 0.0001 Values differ significantly at p<0.05.

Dual fermentation: a, Fermentation flasks with control and test; b, standard curve of ethyl alcohol and c, ethyl alcohol activity (U/ml) in YPS medium, control: inoculated with *Saccharomyces cerevisiae* and test inoculated with *Saccharomyces cerevisiae* & *Brevibacillus borstelensis* R1

In dual fermentation by using co-strains, *Brevibacillus borstelensis* R1 (α-amylase producing) along with *Saccharomyces cerevisiae* gave the best results on 21st day in ethyl alcohol production than with *S.cerevisiae* alone. Similarly dual fermentation with *Bacillus spp.* in ethyl alcohol production was reported by Matsumoto *et al.*[13], De Moraes *et al.*[11], Moraes *et al.* [17], Santa Maria *et al.* [18], Öner [19] and Chi *et al.*[9].

CONCLUSION

The production of ethyl alcohol was estimated and analyzed for 30 days at regular intervals. Yeast extract, peptone and starch medium (YPS) was inoculated with *Saccharomyces cerevisiae* as control and with *Saccharomyces cerevisiae* and *Brevibacillus borstelensis* R1 as test. In our studies of dual fermentation, 5% co-strains of *Saccharomyce cerevisiae* and *Brevibacillus borstelensis* R1 showed maximum (3.37±0.23U/ml) alcohol production on the 21st day.

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