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Starch as precursor of bioethanol production through rdr baker's yeast, *Saccharomyces cerevisiae*

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Abstract- In bioethanol production saccharification of biomass to fermentable sugar is an important constraint because the enzyme production costs more and the complications associated with the removal of hearse acid, alkali and salts formed after neutralization. This led to the search for low cost enzyme and its combination with dilute acid to enhance biomass hydrolysis. In this study, the biomass of microalgae was hydrolysed using amylase and cellulase enzymes produced by solid state and submerged fermentation processes. Dilute tetraoxosulphate (VI) acid, crude enzyme complex and a combination of both were used to study saccharification of algal biomass. The highest yield of reducing sugar of 0.63 mg/ml was obtained with the co-combination hydrolysis of acid and enzyme, followed by acid hydrolysis (0.41 mg/ml) while the least was found with enzyme hydrolysis (0.36 mg/ml).

Keywords- Yeast, optimization, ethanol, starch, fermentation, nuclear petite

INTRODUCTION

Sources of alternative energy have attracted researcher's interests to focus on biomass as a cost efficient and effective feedstock for bioethanol production and can serve as a suitable transportation fuel replacing the limited crude oil[1]. The production of alcoholic beverages from fermentable carbon sources by yeast is the oldest and most economically important of all biotechnologies. Yeast plays a vital role in the production of all alcoholic beverages and the selection of suitable yeast strains is essential not only to maximise alcohol yield, but also to maintain beverage sensory quality. *Saccharomyces cerevisiae* strain of yeast dominates in the production of alcoholic beverages worldwide, and the particular strains of this species employed in fermentation exert a profound influence on the aroma and flavour characteristics of different beverages.[2,3]

Pure cultures of selected strains of *S. cerevisiae* are usually used for large-scale beverage fermentations, as in brewing, winemaking and distilled spirit production. Ethanol is very old chemical and has been made since old times due to the sugar fermentation.[4] Ethanol is produced from any fodder crop which contains simple sugar in abundance or their polymers.[5] The polymers like starch and cellulose are broken down into simple sugars through chemical hydrolysis or enzymatic hydrolysis (saccharification), and then converted by fermentation process to ethanol and carbon dioxide[6], in saccharification, process starch is

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converted into simple sugar (monosaccharide) using microorganism or enzymes such as glucoamylase and α-amylase.[7]

A two-step process includes the bioconversion of starch into ethanol. The first step is saccharification, where starch is converted into sugar using an amylolytic microorganism or enzymes such as glucoamylase and amylase. The second step is fermentation, where sugar is converted into ethanol using *Saccharomyces cerevisiae* .[8]

There are over 150 amylolytic yeast species, their industrial use is limited because of their low ethanol tolerance.[9] So, most of the research is focused on the development of genetically engineered amylolytic strains of *S. cerevisiae*, and in these strains, heterologous genes encoding amylase and glucoamylase from various organisms have been expressed and their products excreted.[10]

Several studies have pointed out the potential of utilizing respiration-deficient nuclear petites for the commercial production of ethanol.[11]

Despite the vast number of strategies adopted for the construction of amylolytic strains of *S. cerevisiae*, there have been no reports about the application of respiration-deficient nuclear petites for the production of ethanol from starch.

In time the development of a respiration-deficient nuclear petite *S. cerevisiae* strain excreting a bifunctional fusion protein that contains both *Bacillus subtilis* amylase and *Aspergillus awamori* glucoamylase activities can be seen. Since the nuclear petite strain generates its energy requirements by fermentation only, lower biomass concentrations and biomass yields from starch were observed with the NPB-G strain as expected.

This study constitutes a first step that provides the basis for utilizing nuclear petite mutants for the single-step bioconversion of starch into ethanol.

MATERIALS AND METHODS

Yeast strains

The 100% respiratory-deficient nuclear petite FY 23pet191 of the parental haploid S. cerevisiae FY23 strain (MATa, ura3-52, trp63, leu21) was generated using polymerase-chain-reaction (PCR)-mediated disruption of the PET191 gene, which encodes a protein required for the assembly of the polypeptide subunits that constitute the active cytochrome coxidase holoenzyme.[12]

Isolation *S. cerevisiae*

The *S. cerevisiae* NPB-G strain was generated by transforming (17) the FY23 pet191 strain with the pPB-G plasmid (5), which contains the *B. subtilis* amylase and the *A*. The *S. cerevisiae* WTPB-G strain was generated by transforming the parental haploid FY23 strain with the pPB-G plasmid and selecting the transformants on yeast minimal medium-agar plates without G418 sulfate.

RESULT AND DISCUSSION

By the formation of starch hydrolysis zones on plates stained with iodine Amylolytic activity was detected. Ethanol fermentation was carried out in an orbital shaker (Innova model no. 4340) at 30°C under aerobic conditions with agitation at 180.rpm. by using shake flask culture NPB-G mutant and WTPB-G parent cells were grown containing YEP-S medium (0.5% yeast extract, 1% peptone, 0.01% uracil, 0.01% tryptophan, 5% starch, 0.4% glucose). Finally the concentration ethanol, glucose and residual plasmid were determined. The highest yield of reducing sugar of 0.63 mg/ml was obtained with the co-combination hydrolysis of acid and enzyme, followed by acid hydrolysis (0.41 mg/ml) while the least was found with enzyme hydrolysis (0.36 mg/ml).

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