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Ethanol production by *Saccharomyces cerevisiae* from rice bran hydrolysates of *Bacillus pimilus* and *Pseudomonas aueruginosa*

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The objective of this work was to study the potential effect of two bacterial xylanase enzymes for ethanol production. The selection of *Saccharomyces cerevisiae* strains to ferment sugars obtained from the rice bran at temperatures above 35^0C with high ethanol yield has become a necessity. In this work *S.cerevisiae* strains were screened for their ability to grow and ferment xylose in the culture filterate produced by *Bacillus pumilus* and *Pseduomonas aueruginosa*. The results obtained from this study showed that rice bran present, an excellent source for agro-residue based ethanol production.

Key words : Rice bran, Ethanol, Yeast, Fermentation

INTRODUCTION

Ethanol production is usually accomplished by chemical synthesis of petrochemical substrates and microbial conversion of carbohydrates present in agricultural products. Owing to the depleting reserves and competing industrial needs of petrochemical feed stocks, there is global emphasis on ethanol production by fermentation process. Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology.

An ideal microorganism used for ethanol production must have rapid fermentative potential, improved flocculating ability, appreciable osmotolerance, enhanced ethanol tolerance and good thermo tolerance. Although no microbial strain has all these desirable qualities, few yeast strains have been found to possess appreciable characteristics for ethanol production (Panchal *et al.,* 1982 ; Hacking *et al.,* 1984).

Various strains of indigenous yeasts capable of producing ethanol have been isolated from different local sources such as molasses (Rose, 1976), sugar mill effluents (Anderson *et al.,* 1986) and local fermented foods (Ameh *et al.,* 1989) and fermented pineapple juice (Eghafona *et al.,* 1999). In most of these studies, the preferred candidate for industrial production of ethanol has been *Saccharomyces cerevisiae.* This yeast also has the ability to produce ethanol which is not contaminated by other products from the substrate. Rice bran is readily available agricultural waste with abundant carbohydrate content and other basic nutrients that can support yeast growth. In the present paper, utilization of xylanase containing xylose produced from *Bacillus pumilus* and *Pseudomonas aueruginosa* was used as the substrate for the growth of *Saccharomyces cerevisiaceae* and production of bioethanol.

MATERIALS AND METHODS

Yeast strain and media :

The yeast used in these studies was *Saccharomyces cerevisiae* known as bakers yeast was purchased from Sakthi Sugars, Coimbatore. The yeast culture was maintained in medium contained 20g of glucose, 20g of agar, 5g of peptone, and 5g of $MgSO_4.7H_2O$ per liter. The growth medium utilized in the liquid inoculation contained 50g glucose, 5g of yeast extract, and 1g of KH_2PO_4, 0.3 g of NH_4Cl and 2g of $MgSO_4.7H_2O$ per liter.

Inoculum preparation :

Cell suspension (10ml) prepared from 2 days old slant culture was inoculated into 100ml of medium and incubated at 30^0C for 48h on a rotary shaker. The cells were then collected by centrifugation. The inoculum concentration of 0.1 per cent (dry weight/volume) was used for the fermentation process.

Production of ethanol from agro residues (Rice bran):

Ethanol production was carried out in two steps (1) Saccharification of agro residues by bacterial enzymes (2) Fermentation of sugar rich hydrolysates obtained from the saccharification process by *Saccharomyces cerevisiae.*

Saccharification :

The agro-residue rice bran rich in lignocellulosics was used as substrate for ethanol production.

Substrate pretreatment :

The substrate was pretreated with 1N NaOH for 1h at 100^{0}C.The pretreated substrate was washed thoroughly with distilled water, dried at room temperature and stored in a airtight container.

Saccharifying enzyme production :

Horikoshi II medium amended with pretreated substrate (1% w/v) was inoculated with bacterial strain and incubated in orbital shaker (125 rpm) at 30^{o}C for 5 days. After 5 days, the culture broth was filtered and centrifuged at 10,000 rpm for 20 min at 4^{0}C.The clear supernatant was used as saccharifying enzyme and analyzed for xylanase activity.

Saccharification of substrate :

The enzyme preparation was added to the pretreated substrate suspended in 0.05m, pH 5.0, sodium citrate buffer (1%w/v) at a concentration of 5.26 IU xylanase activity per ml of suspension and incubated at 50^{0}C for 72 h at 100 rpm.After enzyme addition the yeast inoculum from the selected strain was added.

Fermentation process :

Separate hydrolysis and fermentation process :

In this process, the hydrolysates obtained from saccharification processes were amended with the components of the production medium except that rice bran and fermented by *Saccharomyces cerevisiae* (0.1%w/v) for 72h at 30^{0}C.Ethanol produced was estimated at regular time intervals of 12h by the method of Caputi *et al*. (1968).

Simultaneous saccharification and fermentation :

In this process, the saccharification and fermentation were performed in the same vessel at 30^{0}C. Samples were analyzed for ethanol production at regular time intervals of 12h for 72h.The theoretical SSF yield was calculated by assuming that all the potential xylose in the pretreated material was available for fermentation. The experiments were performed in triplicate.

Ethanol estimation :

An a aliquot volume (1ml) of the sample was made up to 25ml and distilled at 78^{0}C.The distillate 3ml was collected in a flask containing 25 ml of chromic acid and made up to 50 ml of chromic acid and kept in a water bath at 60^{0}C for 30min.After 30 min, the contents were cooled and the colour intensity was measured at 600nm in calorimeter. Calibration curve was drawn using ethanol as standard.

Ethanol yield :

In separate saccharification and fermentation process, while testing the fermentation broth for ethanol content the initial and final residual reducing sugar content of the broth was determined (Miller, 1959).The difference between the initial and final reducing sugar content was interpreted as reducing sugars utilized (RSU).The ethanol yield was then calculated by the modified formula proposed by Gunasekaran and Kamini (1991).

Ethanol yield = Ethanol produced x 100/Reducing sugar utilized.

GC Chromatographic analysis :

GG analysis of fermentation broth to estimate the ethanol content was carried out using GC instrument with FID detector. This experiment was carried out under the following operating conditions.

Instrument used:

Aglient technologies: GC6890N, Detector: FID

Temp ramp:

45^{0}C-4 min; 10-100^{0}C-1minhot, 15-2000C-10min, Total run time: 29.17sec, Injection volume: 0.2 µl, Column used: DB 5ms.30mx0.25 mid x 0.2µ flim thickness.

RESULTS AND DISCUSSION

In the present study hydrolysates obtained by *Bacillus pumilus,* xylanase was 2.212g/l, in *Pseudomonas aueruginosa* hydrolysates it was 2.417g/l. Ethanol yield in these hydrolysates were 58.08 and 60.08 %, respectively (Table 1). These results revealed that the *Pseudomonas aueruginosa* hydrolysates was good for saccharification of rice bran. The culture filterate was analyzed in gas chromatography (GC) to find out the percentage of ethanol by comparing it with standard ethanol. The results of showed 0.28% of ethanol in *Bacillus pumilus* and 0.27 % in *Pseudomonas aueruginosa*. In these results *Bacillus pumilus* was found to produce maximum percentage of ethanol.

Hari Krishna *et al.* (2001) have reported ethanol yield of 2-2.5 %(w/v) in 72h SSF of lignocellulosic wastes with thermo tolerant yeast at 10 %(w/v) initial substrate concentration. Microwave alkali pretreated straw yielded 25.8gl-1 ethanol with a yield of 57.5% and alkali pretreated

Table 1 : Ethanol production by *Saccharomyces cerevisiae* from rice bran hydrolysates of *Bacillus pumilus* and *Pseudomonas aueruginosa* (Separate hydrolysis and fermentation)

Incubation period(h)	*Bacillus pumilus*			*Pseudomonas aueruginosa*		
	RSU	EP	EY (%)	RSU	EP	EY (%)
24	2.212	0.05	2.20	2.417	0.08	3.39
48	3.013	1.75	58.08	3.079	1.87	60.08
72	4.014	1.35	33.63	4.312	1.68	38.96
96	3.131	0.95	30.34	3.272	0.89	27.23
144	2.145	0.41	19.11	2.423	0.53	21.87

Initial reducing sugar content of the hydrolysate (g/L)
B.pumilus = 4.526 *P.aueruginosa* = 4.365
Values are means of three replicates; RSU-Reducing sugar utilized (g/L); EP-Ethanol production (g/L); EY-Ethanol yield (%)

straw yielded 23.7gl-1 with a yield of 0.35g/g cellulose under anaerobic conditions (Panagiotou *et al.,* 2005). Addition of xylanase along with cellulase resulted in synergetic effect on ethanol production in SSCF (simultaneous saccharification and co-fermentation) using SAA-treated barley hull and recombinant *E. coli* (KO11). With 3% w/v glucan loading and 4 ml of xylanase enzyme loadings, the SSCF of the SAA treated barley hull resulted 24.1 g/l ethanol concentration at 15 FPU cellulase/g-glucan loading, which corresponds to 89.4% of the maximum theoretical yield based on glucan and xylan (Kim *et al.*, 2008).In the present study, when the pretreated rice bran was subjected to simultaneous saccharification (with enzymes) and fermentation (with *S.cerevisiae*),0.162 g/l of ethanol was obtained after 24h of fermentation in *B.pumilus* and in *P.aueruginosa* 0.185 g/l ethanol, respectively (Table 2). The results of GC analysis showed 1.54 % in *B.pumilus* and 1.78 % in *P.aueruginosa*. In these results *P.aueruginosa* was found to produce maximum percentage of ethanol. From the present study, it can be concluded that the lignocellulosic waste, rice bran can be used as raw material for ethanol production. The alkali pretreatment of the substrate followed by saccharification and fermentation could yield high amounts of ethanol.

Table 2 : Ethanol production from rice bran by simultaneous saccharification and fermentation (SSF) by *Bacillus pumilus* and *Pseudomonas aueruginosa*

Incubation period(h)	*B. pumilus*		*P.aueruginosa*	
	EP%	EPS%	EP%	EPS%
24	0.245	0.024	0.265	0.026
48	1.623	0.162	1.854	0.185
72	1.548	0.158	1.798	0.179
96	1.356	0.135	1.623	0.162
144	1.356	0.135	1.623	0.162

EPS-Ethanol production g/g substrate; Initial concentration of the substrate 1% (w/v).

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