JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2011. Vol. **5**(1), p. 421-424

Utilization of *Atrocarpus heterophyllus* Lam. (Jack fruit) Seeds as a Substrate for Bio-ethanol Production

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(Received: 07 October 2010; accepted: 19 November 2010)

With an increasing demand for energy, alternate sources other than the conventional resources need to be explored. Bio-ethanol seems to be a promising substitute. Hence, an attempt was made to use jackfruit (*Atrocarpus heterophyllus*. Lam) seeds as a substrate for ethanol production. Amylolytic bacteria were isolated from different sources like carrot, tomato, potato, farm yard manure, forest litter and vermicompost. These isolates were used to saccharify the complex polymers to release fermentable sugars. Further, yeasts were isolated from grapes and distillery effluents and isolates were used to ferment sugars to yield ethanol.

Amylolytic bacterial isolate from Forest litter (FL_1) yielded the highest quantity of reducing sugars (13.4 g /100g). Yeast isolate from grapes (YG_1) yielded the highest quantity of ethanol (4.3 g /100 g) followed by distillery effluent isolate (YD_1) yielded 3.8 g / 100g. Synergistic effect was observed with dual inoculation of FL_1 and YG_6 resulting in ethanol production of 6.4 g /100g. Hence, dual inoculation with amylolytic and fermentative culture is best option to produce bioethanol.

Key words: Saccharification, Fermentation, Residual sugars, Reducing sugars.

Increase in population demands an increased supply of energy. The conventional energy resources like natural gas, coal, oils *etc*., are being depleted at an alarming rate and in a few years these resources may get exhausted. With the inevitable depletion of the world's energy supply, there has been an increasing worldwide interest in alternative sources of energy (Lynd, 1996).

Use of renewable energy is one of the most efficient ways to achieve sustainable development Ethanol is one among the renewable energy sources. The current ethanol production process using crops such as sugarcane and corn are well established. However, utilization of cheaper substrates such as starch and lignocellulosic substrates for ethanol production could make bioethanol more competitive with fossil fuel (Nigam, 1999). Two of the most important issues relevant to conversion of carbohydrate to ethanol are the cost and the availability of the substrate. It is necessary to develop an economical process which allows the use of cheap substrates, such as starch conversion into fermentable sugars and further into ethanol. Among many starchy materials, cassava starch is an inexpensive fermentable source. However, processing of cassava waste is difficult because of the presence

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of cyanogenic glycosides, which on hydrolysis yield hydrogen cyanide. Hence, an alternate cheap starchy material for ethanol production is the need of the hour.

Jackfruit (*Atrocarpus heterophyllus* Lam.) is a monoecious evergreen tree that is popular in several tropical countries. Seeds make up around 10 to 15 % of the total fruit mass. Sanjeev kumar *et al*. (1988) reported that jackfruit seeds are rich in starch with starch content up to 15 %. These seeds do not find much use. Hence, the main objective of this study was framed as the utilization of jackfruit seeds as a substrate for ethanol production. Industrial process for starch hydrolysis relies mostly on enzymatic hydrolysis whose optimum temperature usually ranges from 50° C to 70° C (Satyanarayana *et al*., 2004). Use of enzymes for release of fermentable sugars is an expensive process. So, substitution of enzymes with amylolytic isolates appears to be cost effective process to release maximum fermentable sugars from starch rich substrate. Once fermentable sugars are released from the substrates, they can be further fermented to ethanol by yeasts. Hence, in this study amylolytic bacteria were isolated from different sources and were used to saccharify jackfruit seeds and sugars that are released were further fermented to ethanol using yeast isolates. Further, studies on ethanol production by combined inoculation of starch degraders and yeasts were also conducted.

MATERIAL AND METHODS

Amylolytic bacteria were isolated from different sources *viz*., carrot, farm yard manure, tomato, forest litter, vermicompost and potato. Yeasts were isolated from grapes and distillery effluent. *Saccharomyces cerevisiae* 101 was used as reference yeast for fermentation. All the amylolytic bacterial isolates were screened for amylolytic activity. Starch broth was prepared with starch at 10 mg /ml concentration and was inoculated with 24 hour old culture of amylolytic isolates at 2.5 % and incubated at 32^0 C. Reducing sugars were estimated at regular intervals *viz*., 12, 24, 48 and 72 hours. Efficient isolates were further used in saccharification of substrate. Six gram of jackfruit seed powder was taken in 100 ml flasks and 60 ml of distilled water was added, and sterilized in an autoclave at 121^{0}C for 15 minutes. Flasks were inoculated with efficient amylolytic isolates and incubated at 32^0 C. Saccharification was allowed for 48 hours. Reducing sugasr and total sugars were estimated using DNSA (dinitro-salicylic acid) method.

Substrate was saccharified using an amylolytic isolate that showed the highest reducing sugars release in 1. 5 liters (150 g of powdered jackfruit seed dispersed in 1.5 litres of distilled water in a 2 litre flask) for ethanol production using yeasts. Reducing sugar content was estimated after Saccharification. Saccharified substrate (100 ml) was distributed in flasks and sterilized again. Yeast isolates were inoculated and incubated for 24 hours followed by anaerobic condition maintained by using a cork and provision for CO_2 evolution was provided. Residual sugars and ethanol contents were estimated after five days of fermentation

Dual inoculation of starch degraders and yeasts for ethanol production was studied. Jackfruit seeds powder (10 g) added to 100 ml of distilled water was sterilized and amylolytic bacteria and yeast isolates (24 hour old culture) were co-inoculated and incubated for 48 hours. An anaerobic condition was maintained after 48 hours using a cork and provision for CO_2 was provided. Ethanol content and residual sugar contents were estimated after five days of fermentation.

RESULTS AND DISCUSSION

Starch degrading bacteria were isolated from different sources like carrot, tomato, farm yard manure, forest litter, vermicompost and potato. Colony morphology, Gram reaction and cell shape of isolates are presented in Table 1. Most of the isolates were Gram positive cocci and a few Gram positive and negative rods.

Amylolytic activity of the bacterial isolates is presented in Table 2. Quantity of reducing sugars released is correlated to the amylolytic activity of bacterial isolates. There was a significant difference among the isolates with respect to release of reducing sugars. Isolate FL_1 yielded the highest reducing sugars (4.85 mg / ml). Among the different duration of incubations, 48 hours was found to have the highest amylolytic activity. There was reduction in the reducing sugar

Table 1. Amylolytic bacterial isolates from different sources and their characterization.

Isolates	Source	Colony Morphology	Gram reaction	Cell shape
CaB	Carrot	Raised, dull white colonies	Positive	Rod
FYM1	Farm Yard Manure	Raised, white colonies	Positive	Rod
FYM2	Farm Yard Manure	Yellow pigmented, raised colonies	Positive	Cocci
FL1	Forest litter	Yellow pigmented, raised colonies	Positive	Cocci
FL2	Forest litter	Dull white, raised colonies.	Negative	Rod
PB1	Potato	White, raised colonies with irregular margin	Positive	Rod
PB2	Potato	Red pigmented small colonies	Positive	Cocci
VB1	Vermicompost	Raised, white colonies	Positive	Cocci
VB2	Vermicompost	Dull white, raised colonies	Negative	Rod
ToB	Tomato	Dull white colonies with irregular margin	Positive	Cocci

Table 2. Amylolytic activity of bacterial isolates

S. No.	Isolates	Release of reducing sugars mg / ml				
		12hr	24hr	48hr	72hr	Mean
1	FYM2	0.70	2.30	4.10	2.70	2.45
2	FL2	0.90	1.70	2.90	3.30	2.20
3	PB2	0.80	1.80	3.70	2.50	2.20
4	VB1	0.90	2.10	3.40	4.10	2.63
5	VB2	0.90	1.90	3.90	2.30	2.25
6	ToB	1.20	2.90	4.10	2.70	2.73
7	CaB*	1.80	2.80	5.90	5.10	3.90
8	FYM1*	2.40	3.90	6.90	5.20	4.60
9	FL1*	2.30	4.20	7.30	5.60	4.85
10	PB1*	2.10	4.00	6.60	5.10	4.45
Mean		1.40	2.76	4.88	3.86	

Table 3. Release of reducing sugars from jackfruit seeds by amylolytic bacterial isolates

Isolates	Reducing sugar yield (g / 100 g)	% increase over control
CaB	12.2	154.16
FYM1	12.8	166.66
FL1	13.4	179.17
PB1	12.6	162.5
Control	4.8	-

yield after 48 hours. This might be because of bacterial isolates utilizing sugars released for their growth.

The quantity of reducing sugars released from jackfruit seeds are presented in Table 3. There was a significant difference among reducing sugar yields by different isolates. Isolate FL_1 yielded the highest reducing sugar (13.4 g / 100 g) followed by FYM_1 (12.8 g / 100 g). Least reducing sugar yield (4. 3 g / 100 g) was in control treatment.

The results relating to ethanol production from saccharified liquor prepared from jackfruit seeds are presented in Table 4. Yeast isolate YD_1 and YG_6 fermented the saccharified starch producing 4.3 g and 3.8 g ethanol respectively. The highest ethanol production (4.3 g / 100g of substrate) was obtained by yeast isolate $YG_{6.}$ The other isolate YD_1 and reference yeast strain were on par in action. Similar results have been reported by Kobayashi *et al*., 1998, for ethanol production from potato (4.2 g / l).

Consortiums were developed using the results of saccharification and fermentation.

Table 4. Ethanol yields using yeast strains from saccharified substrates

S. No	Yeast isolates	Initial reducing sugar (g / 100 g)	Ethanol content (g / 100g)	Residual sugars (g / 100 g)
1	YD1	13.0	3.8	4.2
2	YG6	13.0	4.3	3.3
3	*Saccharomyces cerevisiae* 101	13.0	3.1	4.8

Table 5. Ethanol yields by dual inoculation of starch degraders and yeast

S. No	Isolate combination	Ethanol content g / 100 g of substrate	Residual sugars g / 100 g of substrate
1	CaB + YG6	4.2	2.7
2	FYM1 + YG6	4.8	3.0
3	FL1 + YG6	6.4	3.4
4	PB1 + YG6	4.5	2.7
5	YG6	1.2	2.5
6	Control	ND	4.5
	S.Em ±	0.05	0.06
	CD	0.16	0.18

Efficient starch degraders *viz*., CaB, FYM_1, FL_1 and PB_1 and efficient yeast isolate YG_6 were used for consortium preparation. Ethanol production from jackfruit seeds by combined inoculation of starch degrader and yeasts are presented in Table 5. Yeast YG_6 with bacteria FL_1 produced the highest ethanol compared to other bacterial associates.

Thus the present study indicates that jackfruit seeds which are rich in starch can be used as an alternate substrate for ethanol production. The results also revealed that ethanol production by combined inoculation of starch degraders and yeasts yield comparatively higher ethanol than the two single stage processes *i.e*., saccharification and fermentation.

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