Journal of
Microbial & Biochemical Technology

Jaiswal et al., J Microb Biochem Technol 2016, 8:6
DOI: 10.4172/1948-5948.1000327

Research Article Open Access

Bioethanol Production by Novel Indigenous Yeast Strains from Lignocellulosic Waste

Jaiswal Alok, Deepa Tomer and Bhatnagar Tripti*

Codon Biotech Pvt. Ltd., Noida, UP, India

Abstract

The present study deals with isolation of novel indigenous yeast strains and their use in conversion of lignocellulosic agricultural and household waste into bioethanol. 11 yeast cultures were isolated from different sources and from these four strains of yeast were selected on basis of bioethanol production. Biochemical tests were performed to characterize the isolated yeast cultures. The identification of the unknown yeast strains was then done using the 26S rRNA gene sequence analysis. The yeast cultures were identified as: *Pichia farinose*, *Arxula adeninivorans*, *Rhodotorula colostri*, *Stephanoascus ciferrii*. These strains were inoculated in fermentation media having lignocellulosic household and agricultural waste in a 3 L fermenter. After 48 h all the four yeast strains converted the lignocellulosic waste to different amounts of bioethanol. *Pichia farinose* was found to produce a maximum amount of bioethanol, i.e., 31 g/L and *Stephanoascus ciferrii* produced 28.73 g/L bioethanol when bagasse was used as the carbon source.

Keywords: *Pichia farinose*; *Arxula adeninivorans*; *Rhodotorula colostri*; *Stephanoascus ciferrii;* Bioethanol; Lignocellulosic wastes

Introduction

Global concerns about climatic changes and the resultant need to reduce greenhouse gas emissions have encouraged the use of bioethanol as a gasoline replacement or as an additive [1]. Increase in the world's energy demand and the progressive depletion of oil reserves have motivated the research for alternative energy resources, especially those energy resources which are derived from renewable materials such as biomass [2]. The Indian Government has enforced and then deferred the 5% mandatory bioethanol blending law a couple of times since 2003. Currently, as per the policy document, the mandatory blending requirements stand at 10%. However, the ground reality is that the Oil Marketing Companies have not been able to procure bioethanol necessary at the predetermined contract prices.

At present, apart from sugarcane, molasses and grains; no other viable biomass to bioethanol technology exists in India. Many investigators have studied the production of bioethanol using only *Saccharomyces cerevisiae* with different raw materials such as agricultural wastes, municipal wastes [3], the fruit wastes like papaya [4] banana peels [5], pineapple [6] and grapes [7]. It is also been well established that bioethanol can be produced by different classes of yeasts. Different yeast strains would result in different levels of productivity of bioethanol. Thus, bioethanol production depends on microbial activity, particularly that of yeasts.

The present study is an attempt to analyze the conversion of lignocellulosic waste into bioethanol using novel and natural indigenous yeast strains which are commonly founded in sugar rich environments. The yeasts strains were isolated and selected based on their bioethanol producing capacity from lignocellulosic wastes. Initially 11 yeast strains were isolated. Out of these 11 strains, 4 strains resulted bioethanol production. These four yeast strains were further identified and characterization using biochemical tests and 26S rRNA gene sequence analysis.

Materials and Method

Isolation and biochemical characterization of yeast cultures

Samples of locally available curd, juice samples and soil samples around the juice vendors shops was collected. From these samples yeast were isolated using malt agar media, which is a selective media [8]. Initially 11 isolates of yeast were obtained on the selective media (malt agar) plates. These yeast strains were then inoculated in fermentation media having glucose to screen for ethanol production. To characterize and identify the yeasts different Biochemical tests were performed. Urease test, carbohydrate utilization test, nitrate reduction test, survival on 10% NaCl and 16% NaCl, growth in SIM medium, citrate test, survival at different temperature, etc. were used for characterization. The yeast strains were further identified using 26S rRNA gene sequence analysis and BLAST comparison online.

Fermentation media for ethanol production

Initially the 11 yeast strains were screened for ethanol production using 200 g/L dextrose, 5 g/L yeast extract, 10 g/L urea in the fermentation media. The lignocelluloses waste material was collected from kitchens of college hostels and juice vendors. The waste material was then hydrolyzed overnight using 8 M sulphuric acid. After overnight hydrolysis the waste mixture was neutralized and pH was adjusted to 5.5 pH. The fermentation media was prepared by addition of 200 g of dried and powdered lignocellulosic waste, 5 g yeast extract, 10 g urea and 50 g dextrose in 1000 ml of fermentation media. The lignocellulosic waste materials used were: bagasse powder, orange peel and pulp, kitchen lignocellulosic waste and wheat straw waste. The fermentation media was completely sterilized by autoclaving for 15 min at 121°C. 10% yeast cultures was then inoculated in the fermentation broth. Batch fermentation was carried out in a 3 L fermenter with the various specifications as follows: agitation: 150 rpm, pH: 5.5, temperature: 27°C for 72 h under anaerobic conditions.

***Corresponding author:** Bhatnagar Tripti, Codon Biotech Pvt. Ltd., Noida, UP, India, Tel: 0120 431 1464; E-mail: tripti.codonbt@gmail

Received October 01, 2016; **Accepted** October 25, 2016; **Published** November 03, 2016

Citation: Jaiswal A, Tomer D, Bhatnagar T (2016) Bioethanol Production by Novel Indigenous Yeast Strains from Lignocellulosic Waste. J Microb Biochem Technol 8: 474-477. doi: 10.4172/1948-5948.1000327

Copyright: © 2016 Jaiswal A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Analytical methods

The ethanol produced was estimated by gas chromatograph (Nucon) using packed column with FID (flame ionization detector) detector GC-system had a 6 ft. Porapak N packed column (80/100 mesh) and N_2 as the carrier gas. Oven, injector, and detector temperatures were 80, 110 and 110°C, respectively. External standard was applied for identification and quantification. Ethanol was determined in g/L. The peak eluted was noted (using WINACDS 6.2 software) and by knowing the area of peak, the concentration of ethanol was calculated using calibration chart. Total reducing sugar was measured by the dinitrosalicylic acid (DNS) method [9].

Results

The lignocellulosic waste was obtained from juice vendors and from hostel kitchen. These were the wastes which were collected and then were dried by keeping in the sunlight. The dried waste was then powered for further use. Initially yeast strains were isolated on Malt agar plates. 11 yeast strains were isolated and were screened for ethanol production using only dextrose as carbon source (Table 1). The strains which showed ethanol production were then characterized and identified using Biochemical tests and 26S rRNA sequencing.

The results of physical morphology and biochemical tests of the yeast colonies on malt agar are depicted in Tables 2 and 3.

Screening of the isolated yeast strains showed different levels of bioethanol production in all cases when dextrose was used as a carbon source. From these strains four yeasts were seen to produce very high amount of bioethanol. The strain no. 2, 3, 5 and 10 showed around 40 g/L production. These were then given the names Culture No. 1, 2, 3 and 4, respectively while proceeding for biochemical and sequencing analysis.

Depending upon the biochemical tests, sugar utilization results and sequence analysis of the D1/D2 region of the large-subunit ribosomal of 26S rRNA gene, identification of the unknown yeast strains was done on basis of similarity index after comparison on BLAST. The results showed that:

- Culture 1 - *Pichia farinose* (93.33%)
- Culture 2 - *Arxula adeninivorans* (93.33%)

Yeast strain	Bioethanol gm/L after 24 h	Bioethanol gm/L after 48 h
# 1	0.12	0.83
# 2	20.05	45.21
# 3	17.56	41.37
# 4	0.52	1.78
# 5	15.21	35.42
# 6	1.24	2.85
#7	0.27	1.76
# 8	1.30	3.48
# 9	0.86	2.55
# 10	18.50	42.23
# 11	1.08	3.16

Table 1: Bioethanol production obtained during screening of yeast strains.

Name of culture	Color of colony	Surface of colony
Culture-1	White	Rough
Culture-2	White	Rough
Culture-3	Off White	Smooth
Culture-4	White	Smooth

Table 2: Malt agar results of isolated Yeast culture.

S. No.	Types of biochemical test		Culture 1	Culture 2	Culture 3	Culture 4
1.	Biochemical Test	Citrate	+	-	+	+
2.		Nitrate	+	+	+	-
3.		10% NaCl	+	+	+	+
4.		16% NaCl	+	+	+	+
5.		Urease	-	-	+	-
6.		SIM medium (H2S)	+	-	-	-
7.	Carbohydrate Test	Glucose	+	+	+	+
8.		Mannitol	+	+	+	-
9.		Sucrose	+	+	+	+
10.		Fructose	+	+	+	+
11.		Glycerol	+	+	+	-
12.		Maltose	+	+	+	-
13.		Starch	-	-	-	-
14.		Lactose	-	+	+	-
15.	Growth at different temperatures	27°C	+	+	+	+
16.		37°C	+	+	+	+
17.		45°C	+	+	+	+

Table 3: Biochemical test results of isolated yeast culture.

- Culture 3 - *Rhodotorula colostri* (100%)
- Culture 4 - *Stephanoascus ciferrii* (100%)

Pichia farinosa is now known as *Millerozyma farinose*. It is homothallic diploid yeast. It is a salt-tolerant and osmotolerant yeast strain. *Arxula adeninivoransis* is a temperature dependent strain which has been shown to produce bioethanol during starch fermentation. There is almost no study on the other two strains, i.e., *Rhodotorula colostri* and *Stephanoascus ciferrii,* thus this study gives proof that these isolates may also be involved in bioethanol formation.

The various lignocellulosic waste materials have complex carbohydrate in them which cannot be directly converted to bioethanol by yeast cells. So, overnight hydrolysis of the lignocellulosic waste was performed with sulphuric acid. This resulted in conversion of the complex sugars into simple monosaccharide units or disaccharide units which could be utilized by the yeast strains for conversion to bioethanol (Figure 1).

Gas chromatography was used to estimate and detect the amount of bioethanol produced. It was found that the highest amount of bioethanol was produced on the 2nd day in all of the cases. The concentration of bioethanol produced was obtained as peak area and after which the total amount of bioethanol is calculated in g/L. The results of bioethanol production from lignocellulosic wastes are shown in Table 3 and Figure 2. The bar diagrams are shown to give a comparative picture of bioethanol produced from different types of wastes. The maximum amount of bioethanol produced was 31.93 g/L which was produced from Bagasse by *Pichia farinose* of yeast on second day and *Stephanoascus ciferrii* produced 28.73 g/L bioethanol from bagasse, 15.40 g/L from Orange peel and pulp 10.21 g/L from kitchen wastes and 10.63 g/L from Wheat straw. The highest production of bioethanol was by *Pichia farinose* when inoculated on bagasse. *Pichia farinose* thus proved to be better yeast culture than other yeast cultures. *Stephanoascus ciferrii* was also recognized as important yeast since it produced 15.40 g/L from orange pulp and peel and 19 g/L from kitchen vegetable waste (Table 4).

Discussion

The present study depicts the conversion of lignocellulosic

agricultural and kitchen waste into bioethanol. The process starts with isolation, characterization and identification of yeast cultures and finally the cultures were used to produce ecofriendly and green fuel: Bioethanol. The Yeast cultures were isolated from curd, fermented juice and soil samples around the juice vendor's shops were collected. 11 strains on of yeast was isolated and from these 4 strains of were selected on basis of ethanol production.

The morphology of yeast culture was observed microscopically and different biochemical tests were performed to prove the identity of the isolated yeast strains. Depending upon the biochemical tests and sugar utilization results and 26S rRNA gene sequence analysis, the identification of the unknown yeast strains was done, wherein Culture 1 - *Pichia farinose* (93.33%), Culture 2 - *Arxula adeninivorans* (93.33%) Culture 3 - *Rhodotorula colostri* (100%), Culture 4 - *Stephanoascus ciferrii* (100%).

Lignocellulose is made up of complex sugars which were first hydrolyzed by acid treatment and then the simpler sugars were converted to bioethanol by the yeast strains. The maximum amount of sugar was produced from hydrolyzed bagasse which is a waste product of sugar cane industry. The released sugars were fermented for 48 h to produce ethanol and estimation of ethanol was done after every 24 h. The maximum amount of ethanol estimated was 30.69 g/L which was produced by fermentation of bagasse by *Pichia farinose* on second day and *Stephanoascus ciferrii* produced 28.7 g/L bioethanol from bagasse, 15.40 g/L from orange pulp waste and 10.2 g/L vegetable waste .

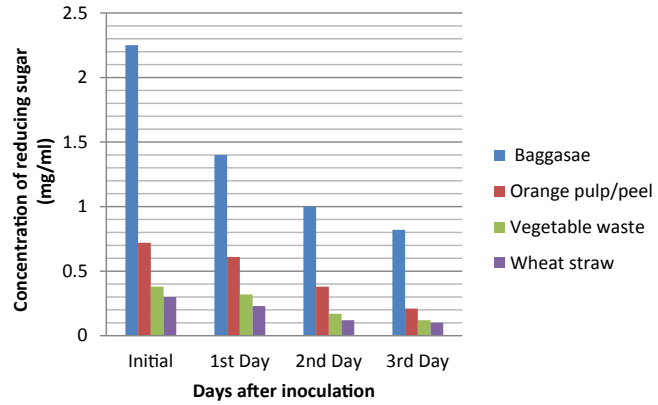

Figure 1: A comparative reducing sugar estimation in the fermentation broths containing different lignocellulosic wastes.

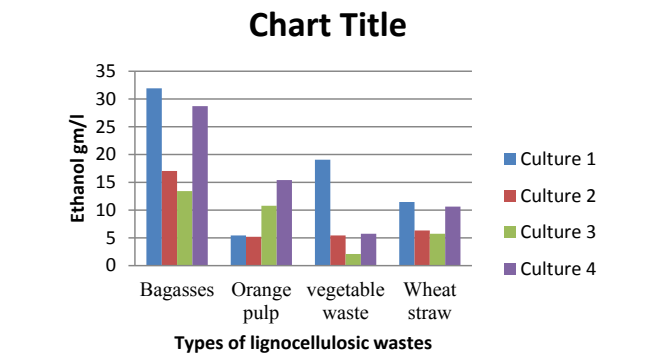

Figure 2: Ethanol production by novel yeast isolates on 2nd day (Culture 1- *Pichia farinose*, 2- *Arxula adeninivorans*, 3- *Rhodotorula colostri*, 4- *Stephanoascus ciferrii*).

Concentration of reducing sugar in different samples after acid hydrolysis (mg/ml)				
Samples	**Initial conc.**	**After 24 h**	**After 48 h**	**After 72 h**
Bagasse	2.25	1.4	1.0	0.82
Orange pulp/peel	0.72	0.61	0.38	0.21
Vegetable waste	0.38	0.32	0.17	0.14
Wheat straw	0.30	0.23	0.12	0.10

Table 4: Concentration of reducing sugar in lignocellulosic waste samples.

In a similar study by Jutakanoke et al. [10], seventy-two yeasts were isolated from sugarcane juices and sugar process-sediments collected in Thailand. These isolated yeasts produced ethanol with 0.46 and 0.22 g/g initial glucose at 30°C and 40°C, respectively. Mishra et al. [11] studied the fermentation of hydrolyzed lignocellulosic waste by *Saccharomyces cerevisiae* under proper incubation conditions to produce bioethanol. Several yeasts are known to assimilate xylose as a source of carbon and convert it into bioethanol. Candida, Pichia, Clavispora, Issatchenkia, Kluyveromyces, Kloeckera, Torulaspora, Geotrichum, Cryptococcus, etc., have been identified for ethanol production from xylose. The studies by Büttner and Bode [12] have showed *A. adeninivorans* which was characterized by industrially important properties and starch utilization for direct conversion of starch to ethanol. All *A. adeninivorans* produced 9-17 g/L ethanol at 30°C and a lesser amount of 0.2-0.5 g/L at 45°C.The ethanol production was achieved by novel species of yeast culture. Another related study by the ethanol concentration reached 9.21 g/L. Another study strongly suggests the possibility of starch-based ethanol production by consolidated bioprocessing using natural yeasts such as *S. shehatae* JCM 18690 [13-16].

In this study novel indigenous and natural yeast showed high bioethanol production from lignocellulosic wastes. *Pichia farinose* and *Stephanoascus ciferrii* gave the highest conversion of sugar to ethanol accumulation in the fermentation media.

Conclusion

It can be concluded that lignocellulosic waste materials such as agriculture waste, sugarcane waste, fruit waste etc. can undergo acid hydrolysis and can be converted to bioethanol. The present study isolation of novel indigenous and natural yeast strains active in high ethanol production would help in industrial production of bioethanol. Increase in the use of bioethanol as a fuel additive can reduce greenhouse gas emission thus reduces air pollution. Bioethanol reduces the dependency on the fossil fuels by reducing the use of petroleum for automobile transportation.

Acknowledgement

The research work has been performed in the laboratories of Codon Biotech Pvt. Ltd., Noida.

References

1. Asli MS (2010) A study on some efficient parameters in batch fermentation of ethanol using *Saccharomyces cerevesiae* SCI extracted from siahe sardasht pomace. Afr J Biotechnol 9: 2906-2912.
2. Balat M, Balat H, Öz C (2008) Progress in bioethanol processing. Progress in Energy and Combustion Science 34: 551-573.
3. Büttner R, Bode R, Birnbaum D (1992) Alcoholic fermentation of starch by *Arxula adeninivorans* , Zentralblatt für Mikrobiologie 147: 225-230.
4. Akin-Osanaiye BC, Nzelibe HC, Agbaji AS (2005) "Production of ethanol from *Carica papaya* (pawpaw) agro waste: Effect of saccharification and different treatments on ethanol yield." Afr J Biotechnol 4: 657-659.

5. Chiranjeevi T, Hari P, Navya A, Praveen C, Veera R, et al. (2013) Isolation and characterization of ethanol tolerant yeast strains 0973-8894 (print) Bioinformation 9: 421-425.

6. Libkind D, Van B (2006) Biomass and carotenoid pigment production by Patagonian native yeasts. World Journal of Microbiology & Biotechnology 22: 687–692.

7. Green M, Shelef G (1989) Ethanol fermentation of acid hydrolysate of municipal solid waste. Che Engg J, p: 40.

8. Ho NW (1998) Genetically engineered saccharomyces yeast capable of effective co-fermentation of glucose and xylose. Appl Environ Microbiol, p: 64.

9. Jimenez J, Benitez T (1986) Characterization of wine yeasts for ethanol production. Appl Microbiol Biotechnol 25: 150-154.

10. Jutakanoke R, Tanasupawat S, Akaracharanya A (2014) Characterization and ethanol fermentation of Pichia and Torulaspora strains. Journal of Applied Pharmaceutical Science 4: 52-56.

11. Manikandan K, Saravanana V, Viruthagiri T (2008) Kinetics on ethanol production from banana peel waste using mutant strain of *Saccharomyces cerevisia*e. Ind J Biotechnol 7: 83-88.

12. Mishra PK (2011) Misregulation of Scm3p/HJURP causes chromosome instability in *Saccharomyces cerevisiae* and human cells. PLoS Genet 7: e1002303.

13. Pramanik, K, Rao DE (2005) Kinetic study of ethanol fermentation of grape waste using *Saccharomyces cerevisiae* yeast isolated from toddy. J Institution of engineers 85: 53-58.

14. Rao RS, Bhadra S (2008) Isolation and characterization of ethanol-producing yeasts from fruits and tree barks. Journal compilation ª 2008 The Society for Applied Microbiology, Letters in Applied Microbiology 47: 19-24.

15. Saxena RC, Adhikari DK, Goyal HB (2009) Biomass-based energy fuel through biochemical routes: A review. Renewable and Sustainable Energy Reviews 13: 167–178.

16. Tanimura A, Kikukawa M, Yamaguchi S, Kishino S, Ogawa S (2014) Direct ethanol production from starch using a natural isolate, *Scheffersomyces shehatae*: Toward consolidated bioprocessing. Environmental Microbiology Environmental Sciences, Scientific Reports 5: 9593.