International Journal of Advanced Multidisciplinary Research (IJAMR)
ISSN: 2393-8870
www.ijarm.com
Volume 3, Issue 4 -2016

Research Article

SOI: http://s-o-i.org/1.15/ijarm-2016-3-4-7

Isolation and Characterization of Thermophiles Producing Bioethanol

Bhatia Ruchi[1], Deeksha, Bhatnagar Tripti[2*]

[1] Noida International University, Greater Noida., [2] Codon Biotech Pvt. Ltd., Noida
*Corresponding Author: *tripti.codonbt@gmail.com*

Keywords

Thermophiles,
Bioethanol,
lignocellulosic wastes.

Abstract

The emphasis of this work is upon the use of lignocellulosic biomass in production of Bio-ethanol using thermophiles. Production of ethanol from lignocellulosic biomass seems very attractive and sustainable due to its renewable and ubiquitous nature and its non competitiveness with food crops. The result of the present study showed that four strains of Anaerobic Thermophilic bacteria were isolated, identified and characterized from the soil and water sample collected from hot water springs and hot water effluent sites of factories. After initial screening 4 ethanol producing strains were isolated and two strains N2 - *Bacillus vietnamensis* and N3- *Bacillus stearothermophilus* showed higher production of Bioethanol and therefore these two isolates were used to study Bioethanol production using Lignocellulosic waste Baggase (Fermentation media II). It was seen that Strain N2 - *Bacillus vietnamensis* gave maximum production of Bioethanol that is 15 gm/l with Lignocellulosic waste (Bagasse) and 25.05 gm/l when dextrose was used.

Introduction

In the present world scenario, the energy demands are met mostly by the non- renewable energy sources such as oil, coal and natural gas. This has resulted in depletion of these resources, environmental deterioration and public health problems.

Ethanol (Goldemberg, J., 2007) is considered as an alternative renewable fuel with the largest potential to replace fossil fuel. Ethanol has a number of benefits over conventional fuels (Macedo et al, 2008). Ethanol is Carbon neutral, Octane no. is higher with better antiknocking characteristic which helps in increasing the fuel efficiency and greater oxygen content resulting in cleaner combustion. The most favourable aspect is that ethanol can be (Balat et al, 2008) combined with Gasoline and the most common blend is 10% ethanol with 90% Gasoline, which requires no modification in vehicle engine. In India, it is mandatory to blend 5-10% ethanol with Petrol but oil marketing companies (OMCs) are able to mix around 2 per cent only. This is due to the unavailability of ethanol for blending.

Traditionally, (Franceschin et al., 2008, Kim and Dale B, 2002) Bioethanol was produced by microbial fermentation of sugar and starch based crops by *Saccharomyces cerevisiae*, but it resulted in a food versus fuel debate which prevents the use of food crops for ethanol production. Another attractive alternative was microbial fermentation of waste lignocellulosic material by *Saccharomyces cerevisiae* for Bioethanol production. But, *Saccharomyces cerevisiae* is unable to convert the total lignocellulosic biomass into ethanol and thus there is need for isolation of novel microorganisms to help in increasing the ethanol production.

At present, thermophilic (Brock T, 1986) bacteria have gained more attention because of fast growth rate and their ability to degrade a broad variety of both hexoses and pentoses. The present research work thus aims to isolate Thermophilic microorganisms from hot water springs and hot water effluent site of factories and screen them for ethanol production.

Materials and Methods

Sample collection

Soil and water samples from hot springs and hot water effluent sites of factories were collected.

Isolation of Thermophiles

Nutrient agar medium was used for the isolation of anaerobic thermophiles. Serial dilution of the sample which was collected was done and the sample was then spread plated on Nutrient agar medium in anaerobic condition. The plates were incubated at 60°C – 70°C under anaerobic condition i.e. in CO_2 chamber for 24 to 48hrs. Distinctive colonies were picked up and studied further.

Identification and Characterization of the Isolates

The isolated strains were differentiated on the basis of colony characteristic and Gram staining. Different Biochemical test were done and through the Biolog software the anaerobic thermophilic strains were identified. The various biochemical test performed were IMViC test, Growth at 6.5% Nacl and 7% Nacl ,Casein utilization test, Starch utilization test, Urease test, Lecithinase test, Oxidase test, Growth at 65°C, Growth at pH 5.7, Motility test, Nitrate reduction test, Hemolysis, Sugar fermentation test (Fructose, Glycerol, Sucrose, Mannitol, Lactose, Starch, Xylose, Innositol, Maltose, Glucose, Ribose, Arabinose, Sorbitol, Mannose). The isolates were identified initially on the basis of Bergey's Manual of Systematic Bacteriology (Holt et al , 1984).

Preparation of Fermentation Media

To facilitate the production of Bioethanol by the isolated anaerobic thermophilic bacteria a very simple fermentation media was prepared. The components of the fermentation media (I) were Urea (1%), Dextrose (15%), Yeast extract (0.2%). This fermentation was used to initial screen the Ethanol producing strains. Next, Lignocellulosic waste materials were used in the above fermentation media instead of Dextrose. The steps involved in preparation of the Lignocellulosic media were modified from Bjerre et al, 1996 and Azzam 1989.

1. Acid Hydrolysis – Acid hydrolysis was done for the breakdown of lignocelluloses into simple sugars. For hydrolysis 100 ml of 0.2 M H2SO4 was taken and to it 10 gm of baggase was added and kept overnight at room temperature.
2. Neutralization – Neutralization was done using NaOH in order to maintain the suitable pH (6.5-7.0) for the growth of isolated thermophiles.
3. To this neutralized media other components of the fermentation media were added which included Yeast extract 0.2 gm and Urea 1.0 gm.
4. The media was then autoclaved at 15 psi for 15 minutes.
5. To the autoclaved media pinch of Sodium thioglycolate was added to ensure anaerobic condition and the inoculation as well as incubation was done in anaerobic chamber.
6. After inoculation of the fermentation media with different isolated strains, the media was incubated at 60°C for 2 to five days. The ethanol produced was then measured after each day with the help of Gas Chromatograph (Nucon) having packed porapack columns, at 70 °C oven temperature. The ethanol peaks obtained were analysed and tabulated.

Results and Discussion

Isolation of Anaerobic Thermophilic bacteria

In the present study different sample of soil and water from hot springs and hot water effluents of factories were used to isolate anaerobic thermophilic bacteria. Four anaerobic thermophililic chemoautotrphs were isolated from the samples as shown in Table:1

Identification and characterization of Bacterial Isolates

The isolated strains were grown in Nutrient agar medium in anerobic conditions at 50°C under anaerobic condition and Gram staining was done for morphological characterization and further Biochemical test were performed for identification and characterization of the bacterial isolates. The results are presented in Table :1.

Table :1 Morphological and Biochemical test results of the isolated thermophiles

Tests	Strain N1	Strain N2	Strain N3	Strain N4
Gram stain	Gram +ve	Gram +ve	Gram +ve	Gram +ve
Catalase test	+	+	+	+
Indole	--	--	--	--
Methyl Red	+	+	+	+
VP	--	--	--	--
Citrate	--	--	--	--
Growth at 6.5% Nacl	+	+	+	+
Growth at 7% Nacl	+	+	+	+
Casein test	+	+	+	+
Starch test	+	+	+	+
Urease test	--	+	--	+
Lecithinase test	+	+	+	+
Oxidase test	+	+	+	+
Growth at 650C	--	--	--	--
Growth at pH 5.7	+	+	+	+
Motility test	--	+	+	+
Nitrate Reduction test	+	--	+	--
Hemolysis	+	+	+	+
Carbohydrate Tests				
Fructose	+	+	+	+
Glycerol	+	+	+	+
Sucrose	+	+	+	+
Mannitol	+	+	+	+
Lactose	--	--	--	--
Starch	+	+	+	+
Xylose	--	--	+	--
Innositol	+	--	--	+
Maltose	+	+	+	+
Glucose	+	+	+	+
Ribose	--	--	--	--
Arabinose	--	--	--	--
Sorbitol	--	--	--	--
Mannose	--	--	--	--

The isolates were identified initially on the basis of Bergey's manual of Systematic Bacteriology (Holt et al , 1984). The isolated strains were differentiated on the basis of colony characterstics like colour, texture and size of colony and gram stain. By the help of different Biochemical test and through Biolog software the anaerobic thermophiles were identified . The different Biochemical test performed were IMViC, Growth at 6.5% Nacl and 7% Nacl ,Casein test, Starch test, Urease test, Lecithinase test, Oxidase test, Growth at 65^{o}C, Growth at pH 5.7, Motility test, Nitrate reduction test, Hemolysis, Sugar fermentation test (Fructose, Gycerol, Sucrose, Mannitol, Lactose, Starch, Xylose, Innositol, Maltose, Glucose, Ribose, Arabinose, Sorbitol, Mannose).

Finally the isolates were identified as

Strain N1 - *Paenibacillus acidiceler* (83% similarity)
Strain N2 - *Bacillus vietnamensis* (88% similarity)
Strain N3 - *Bacillus stearothermophilus* (85% similarity)
Strain N4 – *Bacillus vietnamensis* (93% similarity)

Production of Ethanol using Fermentation Media (I)

All the strains were inoculated in fermentation media I at 60°C under anaerobic condition and GC was done from 2nd day onwards till the 7th day . It was seen that the strain N2 gave the best result and showed the ethanol production of ± 25.30 gm/l while N3 gave a production of 20.17gm/l of ethanol.

Table :2 Bioethanol production on 7th day in gm/l

S. No	Strains	Ethanol production in gm/l
1	N1	1.70 ±0.7
2	N2	25.30 ± 1.3
3	N3	20.71 ±0.9
4	N4	2.13 ±0.7

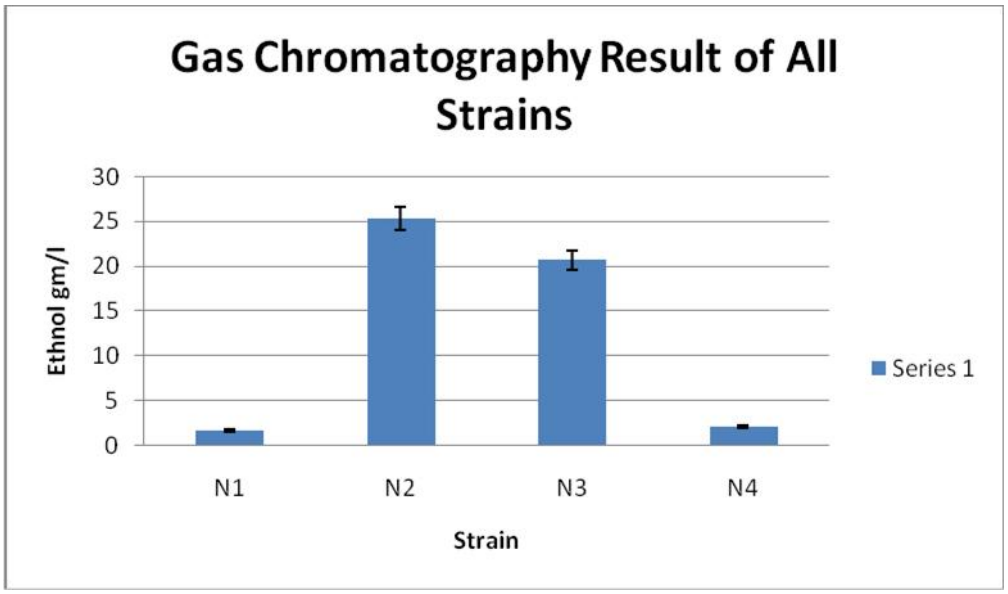

Figure 1: Histogram showing the Ethanol production in gm/l by different thermophiles using Fermentation media (I)

Production of Bioethanol using Fermentaion media (II)

As the strain N2 and N3 showed maximum Bioethanol production with Normal fermentation media (I) these were used to see the production of Bioethanol using lignocellulosic waste Bagasse (Fermentation Media II). Both the strains were inoculated in two sets of Fermentation media II and kept at 50°C in a shaker under anaerobic condition and GC was done from 2nd day onwards till the 9th day. Maximum Production of 15 gm/l Bioethanol by N2 strain was seen on 7th day while N3 strain gave a production of 8.19gm/l± of ethanol.

Table : 3 Bioethanol production from Lignocellulosic waste in gm/l

S. No.	Strains	Ethanol production (gm/l)
1.	N2	15 ± 2.8
2.	N3	8.19 ±1.5

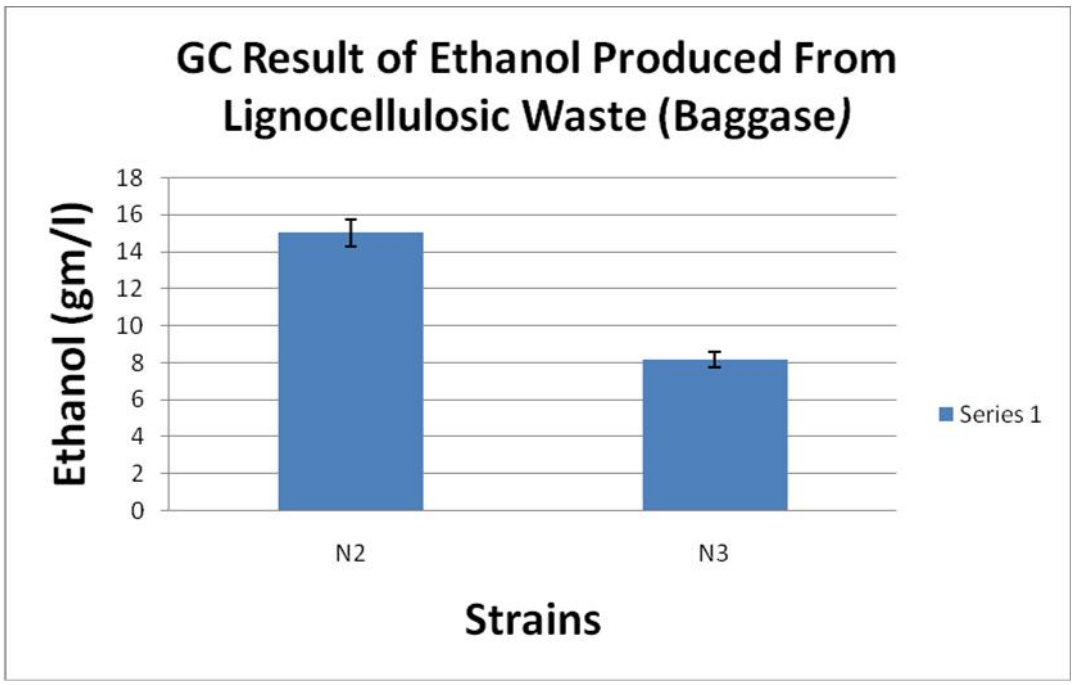

Figure 2: Histogram showing the production of Ethanol when Fermentation media (II) was used.

The result of the present study showed that four strains of Anaerobic Thermophilic bacteria were isolated, identified and characterized from the soil and water sample collected from hot water springs and hot water effluent sites of factories. All of these Four strains N1 - *Paenibacillus acidiceler*, N2 - *Bacillus vietnamensis* , N3 - *Bacillus stearothermophilus* , N4 – *Bacillus vietnamensis* were tested for the production of Bioethanol using simple fermentation media (Fermentation Media I). Out of all the four strains, two strains N2 - *Bacillus vietnamensis* and N3- *Bacillus stearothermophilus* showed higher production of Bioethanol and therefore these two isolates were used to study Bioethanol production using Lignocellulosic waste Baggase (Fermentation media II). It was seen that Strain N2 - *Bacillus vietnamensis* gave maximum production of Bioethanol that is 15 gm/l with Lignocellulosic waste (Bagasse) and 25.05 gm/l when dextrose was used. Thus, with further studies higher ethanol production can be achieved. This ethanol could then be commercially used as additive in fuel.

Acknowledgments

It is certified that all the research has been completed in Codon Biotech's Research Laboratory, Noida.

References

Azzam, A.M.,(1989). Pretreatment of cane bagasse with alkaline hydrogen peroxide for enzymatic hydrolysis of cellulose and ethanol fermentation. J. Environ. Sci. Health. B. 24 (4), 421–433.

Balat, M., Balat, H. and Öz, C., (2008), Progress in bioethanol processing. Progress in Energy and Combustion Science, 34: 551–573.

Bjerre, A.B., Olesen, A.B., Fernqvist, T., (1996). Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysisresulting in convertible cellulose and hemicellulose. Biotechnol. Bioeng. 49, 568–577.

Brock T. (1986). *Thermophiles: General, Molecular and Applied Microbiology*, Introduction: an overview of the thermophiles., John Wiley and Sons, New York. ; 2-15

Cadoche, L., L_opez, G.D., (1989). Assessment of size reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. Biol. Wastes 30, 153–157.

Cardona, C.A. and Sánchez, Ó.J., (2007), Fuel ethanol production: process design trends and integration opportunities. Bioresource Technology, 98: 2415–2457

Franceschin, G., Zamboni, A., Bezzo, F. and Bertucco, A., (2008), Ethanol from corn: a technical and economical assessment based on different scenarios. Chemical Engineering Research and Design, 86: 488–498

Goldemberg, J., (2007), Ethanol for a sustainable energy future. Science, 315: 808–810.

Kim S, Dale B.(2002) Allocation procedure in ethanol production system from corn grain: I. system expansion. International Journal of Life Cycle Assessment; 7(4):237–43.

Macedo, I.C., Seabra, J.E.A. and Silva, J.E.A.R., (2008), Green house gases emissions in the production and use of ethanol from sugarcane in Brazil: the 2005/2006 averages and a prediction for 2020. Biomass and Bioenergy, 32(7): 582–595.

Access this Article in Online	
Quick Response Code	Website: www.ijarm.com
	Subject: Microbial Biotechnology

How to cite this article:

Bhatia Ruchi, Deeksha, Bhatnagar Tripti. (2016). Isolation and Characterization of Thermophiles Producing Bioethanol. Int. J. Adv. Multidiscip. Res. 3(4): 32-37.