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Characterization Of Bacteria For Bioethanol Production From Shorearobusta (Sal) Seeds.

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Abstract

Bioethanol is one of the most interesting biofuels because of its environmental benefits. It is an alternative energy source obtained from food crops, biomass, and algae that is transformed into fuel for motors. It is regarded as a long-term solution to the energy and environmental crisis, as it reduces greenhouse gas emissions and promotes environmentally beneficial technology. *Shorea Robusta* seeds have high carbohydrate content, making them suitable for bioethanol production. The goal of the research is to extract bioethanol from *Shorearobusta* (Sal) seeds. The bacterial strains A. B, C. D, E. F, G and H were screened for their fermentative property through carbohydrate fermentation test. Five of the eight bacterial isolates A, C, D , E, and H were fermentatively positive for bioethanol production. The findings indicate that the highest quantity of bioethanol production attained by bacterial isolate A was 6.9 ± 0.1% (v/v) While as minimum were recorded from bacterial isolate H 3.1 ± 0.1% (v/v).

Keywords: Bioethanol, *Shorea Robusta* seeds, bacterial isolate, etc.

1. Introduction

Excessive usage of fossil fuels, particularly in large urban centers, has resulted in significant levels of pollution over the previous several decades. The concentration of greenhouse gases in the earth's atmosphere has risen dramatically. Global energy consumption has gradually increased as the world's population and economic prosperity have grown. The restricted availability of fossil fuel reserves has an impact on transportation fuel imports. Annual global oil production will start to drop in the near future. In this circumstance, renewable sources could serve as an alternative. (Zabed*et al.,* 2017). Wind, water, sun, biomass, and geothermal heat can all be sustainable energy sources, while fuel production and the chemical sector may rely on biomass as an alternative source in the future. Renewable biomass fuels made from sugarcane, corn, switchgrass, algae, and other sources, such as bioethanol, biodiesel, and biohydrogen, can replace all petroleum-based fuels. Currently, ethanol is the most frequently utilized liquid biofuel for motor vehicles. (Nibedita, *et al.,2012)*.Ethanol is becoming increasingly important for a variety of reasons, including global warming and climate change. Bioethanol has sparked broad interest on the global, national, and regional levels.The worldwide bioethanol market has entered a period of rapid, transitional expansion. Because of decreasing crude oil reserves, several countries are turning their focus to renewable energy sources for power generation. This development is also affecting transportation fuel. Ethanol has potential to be a valuable replacement for gasoline in the transportation fuel industry(Ruan*et al.* 2019).

Bioethanol provides several advantages over traditional fuels, including 35% oxygen, which aids in fuel combustion and decreases particle and NOx emissions (Saini *et al.* 2014). Bioethanol has a higher flammability limit, higher octane number, higher heat of vaporization, and faster flame speed than gasoline, resulting in a higher compression ratio and shorter burn time. This correlates to a higher compression ratio and shorter burn duration in an internal combustion engine (Balat*et al.,* 2008).

Bioethanol production is primarily sourced from starch, sugars, algae, and lignocellulosic biomass. The first generation is produced from starch and sugar, while the second and third generations use lignocellulosic biomass and algae. Third-generation bioethanol from algae is in its early stages and is limited to laboratory studies. First-generation biofuels have a barrier that threatens biodiversity and food supplies, creating a conflict between "food vs. fuel." To avoid this, lignocellulosic biomass, which consists of cellulose, hemicelluloses, and lignin, is an ideal substitute. This method reduces greenhouse gas emissions and is less expensive than first-generation biomass (Halder*et al.* 2019)

.*Shorearobusta* (Sal) seeds contain a high concentration of carbohydrates, making them a possible source of bioethanol production. Shorearobusta, which belongs to the Dipterocarpaceae family, plays a vital role in the economies of Jharkhand, Bihar, Orissa, Chhattisgarh, and Madhya Pradesh. Sal seeds contain fat and triglycerides and are used in goods such as vanaspati, oil, soap, and cocoa butter. *Shorearobusta* (Sal) seeds include 62% carbs, 8% protein, 14.8% oil, 1.4% fiber, 2.3% ash, and 10.8% water. The efficient strains utilized to produce bioethanol have a significant impact on the process. Saccharomyces cerevisiae is the main ethanol-producing bacterium utilized globally. Despite various

advantages, some disadvantages include high aeration costs, high biomass production, and low temperature and ethanol tolerances (Saigal, 1993). Bioethanol can be produced by a variety of bacteria, including Z. mobilis and Klebsiellaoxytoca, as well as fungus such as Trichoderma and Aspergillus sp. Z. mobilis, a gram-negative anaerobic bacteria, has emerged as a promising and unique bacterium for maximum bioethanol production (Dumsday*et al.*, 1997). Z. mobilis has a high specific rate of sugar uptake, high ethanol yield, low biomass production, and does not require controlled oxygen addition to sustain cell viability (Rogers et al., 1980).Ethanol production from lignocellulosic materials begins with the decomposition of the lignocellulosic structure to a fermentable substrate, which is then fermented and distilled to provide 95% ethanol (Olsson &Higerdal, 1996). The present work attempts to produce bioethanol from *Sal* seeds, which are abundant in the Indian state of Madhya Pradesh.

2. Materials and methods

2.1 Collection of Substrates

Shorearobusta seeds were collected from forest area of Betul and Budhni after that they were identified by the Department of Botany J.H.Govt .P.G.College Betul ,(M.P.) India. The seeds were grinded into a fine powder that serves as a feedstock for the synthesis of bioethanol.

2.2 Isolation of bacterial strains from *Shorearobusta*(Sal) seeds during fermentation process:

The serial dilution approach was used to isolate bacteria from *S. rohusta.* The suspensions ranging from 10^{-1} to 10^{-9} were prepared as ten-fold serial dilutions using the fermented sap. Using the spread plate approach, the series 10^{-3}, 10^{-4}, and 10^{-5} were employed to isolate bacteria on nutrient agar media (NAM). Following a 24-hour incubation period, the bacterial species that was isolated was identified based on the appearance of their colonies. Using the streak plate approach, a pure culture of every isolated sp. was created on nutritional agar medium (NAM) plates (Choudhary *et al.*, 2015; Tandon *et al.*, 2018).

2.3 Screening of highest efficient bioethanol producing bacteria

Using the carbohydrate fermentation test, all isolated bacterial strains were evaluated for their fermentative potential. A fermentation medium was made in order to verify the bacteria's capacity to produce gas. Using this procedure, 10g of peptone, 15g of sodium chloride, 5g of carbohydrates, 0.018g of phenol red, 1,000m1 of distilled water, and a pH of 7.3 were used to make the fermentation broth. The indicator employed was phenol red (pH 6.8–7.0), which changes the colour of the medium from yellow to red. In order to detect gas bubbles, Durham tubes were inverted and inserted within tubes containing the fermentation medium. After each bacterial strain was added to the tubes, they were left to incubate for 24 to 48 hours. The change in the colour confirms the acid production character of bacteria and the formation of gas bubble inside the durhum tube confirms the fermentative ability of bacteria (Prescott, 2000).

2.4 Fermentation

Shorearobusta (Sal) seeds by using *Zymomonasmobilis* MTCC 92 (bacteria) was used as a substrate (sole carbon source) for bioethanol production through fermentation process. 20 gm powdered seeds was added in 200 ml of distilled water 10% (w/v) in 250 ml conical flask and then autoclaved it at 121°C, 15 lb pressure for 15 min. (Al-shorgani *et al.*, 2012). The initial pH of *Shorearobusta* (Sal) seed fermentation media was 6. *Zymomonasmobilis* MTCC 92 broth was inoculated with inoculum size of 1% (v/v) separately in *Shorearobusta* (Sal) seed powder under aseptic condition and incubated for 24 hour at 30 °C for fermentation. After fermentation, fermented sample was distilled. For distillation, 25 ml of fermented sample was mixed with 200 ml of distilled water and then poured in the distillation flask and distillation was performed in distillation apparatus (Pharmacopoeia of India, 1985).

2.5 Estimation of Bioethanol

Qualitative estimation for bioethanol

The presence of bioethanol in the fermentation media was analyzed by Jones test (Jones, 1953). For this test, 1 ml of fermented sample was taken in test tube then added 2 ml (2% K2Cr2O7) and 1 ml of conc. H2SO4 then the appearance of bluish green color gave positive result of ethanol and confirms the presence of bioethanol in fermentation media of the micro-organism (Zymomonasmobilis MTCC 92).

Quantitative estimation of bioethanol

Quantitative estimation of bioethanol was done by specific gravity method. Specific gravity refers to the density of any liquid (Pharmacopoeia of India, 1985). Twenty five millilitres fermented sample was mixed with distilled water (make up the volume 150 ml) and this mixture was distilled on distillation unit. After distillation ethanol percentage was calculated by specific gravity method (Yadav, 2003). Percentage in v/v was obtained from the standard table correlating percentage volume of ethanol with specific gravity at 25 °C. Each step was repeated three times. All the values are mean

± standard error, values differ significantly at 5% as analyzed by Duncan multiple Range Test by SPSS. ρ= W3 - W1 X Density of water at t °

$$\rho = \frac{W3-W1}{W1-W3} \times \text{Density of water at t °C}$$

Where
ρ = specific gravity.
W1 = weight of empty specific gravity bottle.
W2 = weight of empty bottle + distilled water, W3 = weight of empty bottle + fermented liquid.
The bioethanol yield (Yp/s) and fermentation efficiency (%) were calculated by using formula:-

$$\boldsymbol{a})\ \text{Ethanol yield (Yp/s), g/g} = \frac{\text{Mass of ethanol formed}}{\text{Mass of sugar consumed}} (\text{Behera } \textit{et al.}, 2012)$$

$$\boldsymbol{b})\ \textbf{Fermentation efficiency}\ (\%)$$

$$= \frac{\text{Ethanol yield obtained (Yp/s)}}{\text{Theoretical maximum ethanol yield from substrate}} \times \mathbf{100}\ (\textbf{Sharma } \boldsymbol{et\ al.}, \mathbf{1975})$$

2. Results

3.1 Isolation of bacterial strains from *Shorearobusta*(Sal) seeds

The morphology of the isolated bacterial species was used to characterize them. The following characteristics of the isolated bacterial colony's cell morphology were analyzed for: colony size, form, elevation, margin, colour, opacity, and number.

The majority of the isolates on NAM were found to be smooth, convex, and pale white with entire margins. A, isolate was circular, pale white, convex, entire and opaque, B isolate was circular, cream convex, entire and opaque,C isolate was irregular, pale white in color, convex, erose and opaque, D isolate was circular, pale white in color, flat, entire and opaque, E isolate was pinheaded, pale white in color, convex, entire and translucent, F was pinheaded, cream in color, flat, entire and translucent, G, isolate was circular, cream, pulvinate, entire and opaque and H, isolate was irregular, yellow in color, pulvinate, irregular and translucent The majority of the isolates on NAM were found to be smooth, convex, and pale white with entire margins (Table 1). Eight distinct bacterial species were identified and coded as A, B, C, D, E, F, G and H.

Table -1:- Showing characteristics of bacterial isolates Colony

S.No.	Bacterial isolates	Shape	Color	Elevation	Margin	Appearance	No. of colonies
1	A	Circular	PALE White	Convex	Entire	Opaque	24
2	B	Circular	Cream	Convex	Entire	Opaque	17
3	C	Irregular	PALE White	Convex	Erose	Opaque	16
4	D	Circular	PALE White	Flat	Entire	Opaque	34
5	E	Pinheaded	PALE White	Convex	Entire	Translucent	12
6	F	Pinheaded	Cream	Flat	Entire	Opaque	9
7	G	Circular	Cream	Pulvinate	Entire	Opaque	4
8	H	Irregular	Yellow	Pulvinate	Irregular	Translucent	31

3.2 Identification of Bioethanol producing bacteria by carbohydrate fermentation test

The bacterial strains A, B, C, D, E, F,G and H were screened for their fermentative property through carbohydrate fermentation test. After the media had been incubated for 24 hours, it was noticed that the colour had changed from red to yellow and that the durhum tubes had risen as a result of the medium producing gas. Five of the eight bacterial isolates A, C, D, E, and H were fermentatively positive (Table-2).

Table -2:-Showing Identification of bioethanol producing bacteria by carbohydrate fermentation test.

S.No.	Bacterial Colony	production of Acid by colony	production of Gas by colony
1	A	Positive	Positive
2	B	Positive	Negative
3	C	Positive	Positive
4	D	Positive	Positive
5	E	Positive	Positive
6	F	Positive	Negative
7	G	Positive	Negative
8	H	Positive	Positive

a. Estimation of Bioethanol productivity Bacterial colony (Qualitative)

Bioethanol was qualitatively estimated for A, B, C, D, E,F and H by Jones reagent test.Ethanol oxidizes to acetic acid with potassium dichromate in the presence of sulphuric acid and gives blue green color. The presence of bioethanol was confirmed by the observation that all five bacterial isolates (A, B, D, E, and H) showed blue-green coloration in the fermented sample (Table-3)

Table-3: Showing estimation of bioethanol by Jones reagent test (Qualitative)

S.No.	Bacterial isolates Colony	Results
1	A	Positive
2	C	Positive
3	D	Positive
4	E	Positive
5	H	Positive

b. Estimation of bioethanol Production (Quantitative)

The five positive fermentative bacterial isolates were screened for highest bioethanol production.The fermentation process were used for the production of bioethanol. Fermented sample was distilled in distillation unit and amount of ethanol produced was calculated by specific gravity method. After a 24-hour incubation period, each bacterial isolate was analysed for the production of bioethanol. The findings indicate that the highest quantity of bioethanol production attained by bacterial isolate A was 6.9 ± 0.1% (v/v) While as minimum were recorded from bacterial isolate H 3.1 ± 0.1% (v/v) (Table-4& Fig- 1).

Table -4: Showing estimation of bioethanol (Quantitative)

S.No.	Bacterial isolates colony	Bioethonal production percentage v/v
1	A	6.9 ± 0.1
2	C	3.4 ± 0.0
3	D	4.7 ± 0.2
4	E	4.2 ± 0.0
5	H	3.1 ± 0.1

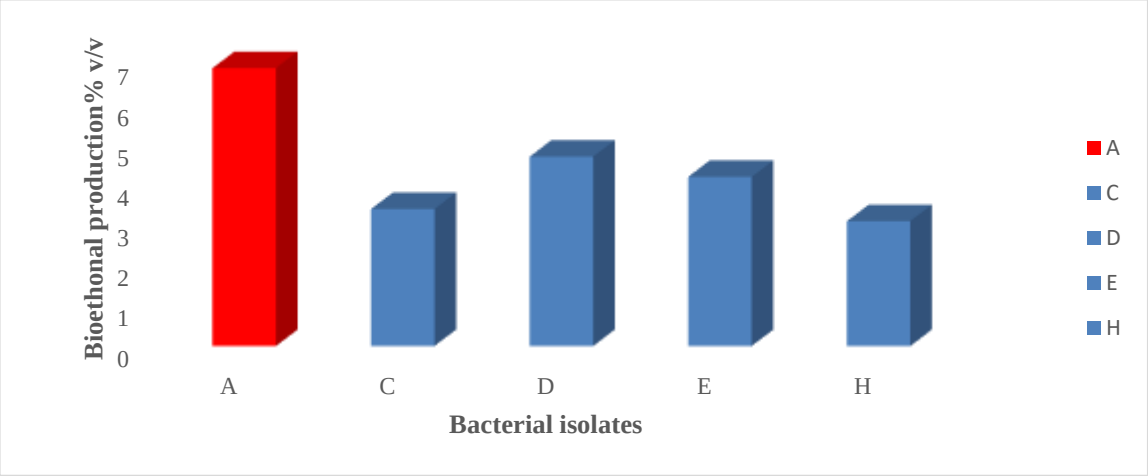

Fig- 1: Showing productionof bioethanol from different Bacterial isolates colony

3. Conclusions

Bioethanol is a promising alternative energy source, derived from food crops, biomass, and algae, which can be used as a fuel for motors. *Shorea Robusta* seeds, with their high carbohydrate content, are ideal for bioethanol production. A study screened eight bacterial strains for their fermentative properties, finding five were positive for bioethanol production. The highest bioethanol production was achieved by bacterial isolate A, with the minimum recorded from isolate H. This research aims to promote environmentally beneficial technology and reduce greenhouse gas emissions.

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