https://doi.org/10.33472/AFJBS.6.10.2024.3911-3921

African Journal of Biological Sciences

Research Paper Open Access

A Study Of Isolation And Characterisation Of Cellulolytic Bacteria For Bioethanol Production And Waste Degradation.

Jaya Philip[1]*, Disha Kaushik[2], Khushi[3], Anjali Mishra[4]

[1]*,[2,3,4]Department of Microbiology, Patna Women's College (Autonomous), Patna

***Corresponding author:** Jaya Philip
*Email:- jayaphilipmicrobio@gmail.com

Article History
Volume 6,Issue 10, 2024
Received: 3-05-2024
Accepted : 19-05-2024
Published : 01-06-2024
Doi:-
10.33472/AFJBS.6.10.2024.3911-3921

Abstract: Cellulose is a key structural component in the cell wall of plants and a complex polysaccharide. This review delves into the diverse array of microbial Marvels that possess the enzymatic system to digest cellulose. Through isolation and characterization, likely isolates were identified for their enzymatic activities crucial in decomposition of cellulose. Partial purification of enzymes was achieved through Ammonium sulphate precipitation. Enzyme activity was assessed using the Dinitro salicylic acid (DNS) method for the presence of enzymes, which is commonly utilized for estimating reducing sugars. The fermentation process employed yeast, specifically Saccharomyces cerevisiae culture, resulting in the production of bioethanol. Through intricate metabolic pathways and synergistic interactions, these microorganisms not only facilitate the production of biofuels like ethanol but also offer a sustainable approach for waste degradation such as paper and textile waste. By integrating microbial ecology with bioprocess engineering, the comprehensive analysis underscores the transformative potential, emphasizing the imperative role of Cellulolytic Crusaders in shaping a greener future. In conclusion bio prospecting from widely existing organic material opens doors to innovative approaches in both waste management and renewable energy production.

Keywords: Cellulolytic bacteria, Cellulase, Bioethanol Production, Paper Degradation

Introduction:

Cellulose is the most abundant biomass composed of glucose units linked by β-1, 4 glycosidic linkages. Cellulose has a prime role in various industries such as pulp and paper industry, textile industry, food industry, pharmaceutical and agriculture industry, for producing important products. Cellulolysis refers to breakdown of cellulose into glucose which can be utilized in production of bioethanol. The three major component of cellulolytic enzyme system are endoglucanases, exoglucanases and β-glucosidases. (Khianngam et al., 2014) Endoglucanase which helps in cleaving of β-glycosidic bonds along a cellulase chain. Exoglucanase works at the non-reducing sugar by cleaving it and helps to split cellobiose. β-glucosidase (β-D-glucoside

glucohydrolase) helps in breakdown of cellobiose to glucose. Hydrolysis of cellulose is mainly done by bacteria and fungi which helps in production of cellulase enzyme for its industrially important applications. The cellulolytic potential of the bacteria determines the enzymatic activity of cellulase enzyme. Bacteria is a dominant cellulolytic microorganism and has good potential for cellulase production. Cellulase enzyme produced by microorganisms plays an efficient role in removal of cellulosic wastes by economical and cost effective methods. (Sethi et al., 2013)

Biofuel such as bioethanol are in high demand in modern world as these can be generated from bioconversion of cellulose. Bioethanol is a renewable fuel with minimal expense. Using agricultural wastes, we can convert cellulose into biofuel which helps to reduce waste and accounts for greenness and environmentally friendly method. (Devi and Munjam, 2022) Bioethanol produced by action of cellulose degrading microorganisms have greater efficiency to replace fossil fuel which are costly and degrades the natural environment thus bioethanol being an alternative fuel for sustainable development. (Thomas et al., 2021)

In textile industries cellulase enzyme helps in breakdown of cellulose enhancing smoothness of fiber and smooth texture. Cellulosic fiber helps to increase strength and sustainability of fibers. Cellulase enzyme also helps in biopolishing of fabrics and leads to breakdown of fibers for stain removal and helps in biomass conversion efficiency. The breakdown of cellulose reduces the fiber damage by specific enzyme reaction thus yielding better quality of the textile material and maintenance of texture. (Korsa et al., 2023)

In paper and pulp industries cellulase enzymes have variety of applications such as strength of fiber is enhanced and enzymatic deinking of paper wastes. By utilizing cellulase enzyme we tend to decrease the cellulosic waste such as agricultural waste, lignocellulosic wastes by economically feasible method by use of cellulose degrading microorganisms. These are also utilized in pulp industries for desired pulp strength and pulp freeness. Cellulase enzymes are also used for production of biodegradable cardboards which can be easily decomposed being environmentally friendly. The decrease in paper wastes utilizing cellulase enzyme is a better alternative than conventional techniques for sustainable development. Demands for cellulase enzyme are increasing due to its potential applications by cost efficient processes and using low energy for improved fabric and paper properties. (Singh et al., 2016)

Material and Methods

Sample collection

Cellulose-degrading microorganisms were obtained aseptically from decomposing sections of woods, compost soil and decaying parts of fruits and vegetables. The collected samples were placed in sterile sample packets and were sealed. The sample packets were transported to the laboratory under sterile conditions and stored at 5℃-10℃.

Enrichment and Isolation of Cellulose-Degrading Bacteria

0.1 gm of decomposing section of wood and compost soil samples were serially diluted in 9.9 ml of sterilized normal saline (0.87%) up to 10^{-5} dilution. 0.5 gm of sample from both the decayed fruits and vegetables was enriched in 50 ml of Carboxymethyl cellulose broth (CMC) and was incubated at 37℃ for 48 hours. The enrichment culture samples were vortexed and mixed thoroughly and 1ml of enrichment culture of both the samples was serially diluted in 9ml of sterilized normal saline (0.87%) up to 10^{-5} dilution.

Carboxymethyl cellulose media (CMC) was prepared, autoclaved and was poured into sterile petri plates under sterile condition. Plates were left for solidification and were inoculated with the

dilution of 10^{-3} and 10^{-4} from all the samples by spread plate method. The plates were then incubated at 37℃ for 48 hours.
Diverse colonies were obtained on CMC plates of different sample and morphologically distinct colonies were isolated by quadrant streaking. Colonies (C1, C2, C3, C4, C5, C6, C7, C8, C9, T10, T11, V12, F13, F14, V15, V16) from all the samples were selected based on the cultural and morphological characteristics and were streaked on Nutrient agar slants and maintained for further use with periodic subculturing.

Detection of Cellulose-Degrading Bacteria

Conformation of cellulose-degrading ability of bacterial isolates was performed by streaking on the Carboxymethyl cellulose plates (CMC) and incubating at 37℃ for 48 hours. The use of Gram's iodine solution as an indicator for cellulose degradation in an agar medium provides the basis for rapid and sensitive screening test for cellulolytic bacteria. Colonies showing discolouration of Iodine solution was taken as positive cellulose-degrading bacterial colonies and only those colonies who have the highest efficiency was taken for further study.
To estimate the cellulose degrading ability of positive isolates, the Z/N ratio was used, calculated by dividing the clearing zone diameter by the colony diameter.

Maintenance of pure culture

The bacterial colonies showing significant clear zone were plated on minimal agar medium (CMC) and analysed for colony characteristics and was sub-cultured on the nutrient agar slants and incubated at 37℃ for 24 hours and then stored at 4℃. (Dsouza and Chandak, 2018)

Morphological and biochemical characterization of cellulolytic bacteria

Morphological analysis

The colony characteristics of the selected bacterial isolates were examined and further utilised for identification. The plates were subjected to Gram staining and microscopic viewing for identification of bacterial strains. This technique was used to distinguish between the gram positive and gram-negative bacteria characterized according to their morphological shape.

Biochemical analysis

The identification of bacterial isolates was carried out through a series of biochemical tests such as the fermentation test, catalase test, citrate utilization test, methyl-red test, Voges-Proskauer test, indole test, oxidase test, motility test, and H2S production test

Extraction of crude enzyme

10ml of Enzyme production medium was taken in a 100ml conical flask. The flasks were sterilized in autoclave at 121℃ for 15 minutes. After cooling, the flasks were then inoculated in aseptic condition with overnight grown bacterial culture of each isolate. The inoculated medium was incubated at 37℃ in a shaker incubator for 24 hours.
With the completion of 24-hour fermentation period, the broth culture was subjected to centrifugation at 5000 rpm for 15 minutes at 4℃. The pellet was discarded and the supernatant was collected and was stored as a source of crude enzyme. (Sethi et al., 2013)

Endo-β -1,4-glucanase activity assay by DNS method

Endo-**β**-1,4-glucanase activity of cellulase was measured by DNS (3,5- dinitro salicylic acid) method through the amount of reducing sugars liberated during hydrolysis.
One unit of Endo β-1,4- glucanase activity was defined as the amount of enzyme that could hydrolyse CMC and release 1µ mol of Glucose within 1 min of reaction. The enzyme assay was carried by three different procedures to ensure best results.

- 1% solution of CMC was prepared in 1 N citrate buffer (pH 5.0) and was considered as substrate. 0.1 mL crude enzymes and 1ml of citrate buffer was added into the mixture of 1mL of CMC solution. The mixture was then incubated at 45℃ for 30 min. To stop the reaction, 3ml of DNS was added to the above solution. After being boiled for 10 mins, the sample was cooled in water to stabilize its colour and then the optical density was assessed at 540 nm (Islam and Roy, 2018)
- 0.5 mL of supernatant was incubated with 0.5 mL of 2% amorphous cellulose in 0.05m sodium citrate buffer (pH 4.8) at 50℃ for 30 min. After incubation, 3mL of 3, 5-dinitrosalicylic acid (DNS) reagent to 1mL of reaction mixture. After being boiled for 10 mins, the sample was cooled in water to stabilize its colour and then the optical density was assessed at 540 nm. (Guder and Krishna, 2019)
- 0.2 mL of crude enzyme solution plus 1.8 mL of 0.5% carboxymethyl cellulose (CMC) in 50mM sodium phosphate buffer (pH 7) was incubated at 37℃ in a shaking water bath for 30min. the reaction was then terminated by adding 3mL of DNS reagent. The colour was ten developed by boiling the mixture for 5 min. Optical density of samples was measured at 540nm against a blank containing all the reagents minus the crude enzyme. (Sethi et al., 2013)

The total CMCase enzymatic activity is defined in international units (IU). One unit of enzymatic activity is defined as the amount of enzyme that releases 1µmol reducing sugars (measured as glucose) per unit per ml.
Enzymatic activity assay of cellulase was calculated according to IUPAC shown in equation below:

$$\text{Enzyme activity (U/ml)} = \frac{(E)(Vf)}{(t)(\epsilon)\ (Vs)}$$

where,
E=Absorbance at 540nm
Vf= final volume of the solution including DNS
Vs= Volume of enzyme
t= incubation tine
d= enzyme dilution
ϵ= extinction coefficient

Partial enzyme purification

The crude enzyme obtained from best bacterial isolates by centrifugation and separation of the supernatant was subjected to 80% Ammonium sulphate precipitation. The procedure was carried forward by mixing a 0.8 mL of 100% saturated ammonium sulphate solution with 0.2 mL of supernatant (crude enzyme). Precipitate was obtained and was allowed to settle at 4℃ overnight. The precipitate was then subjected to centrifugation for 30 minutes at 10,000 rpm. The supernatant was decanted resulting in the collection of pellets in the Eppendorf tube. The pellet

was then re-dissolved with small amounts of ice cold 0.05 M citrate buffer (pH 4.8). (Sulyman et al., 2020)

Bioethanol formation

The mixed culture media with Basal salt medium containing cellulose powder as substrate for production of cellulolytic enzymes was prepared. The addition of cellulose powder initiated the simultaneous Saccharification process. The bacterial isolates were inoculated and allowed to grow in the mixed culture medium.

The culture medium for each isolate was incubated at 37℃ with under shaking condition at 100 rpm for 3 days. After the completion of three days of incubation, the above culture broth was conditioned for Co-culturing of Saccharomyces cerevisae by addition of filter-sterilized salt solution. The simultaneous saccharification and fermentation was carried out at 27℃ for 5 days in a stationary condition. After the required fermentation period, qualitative analysis of bioethanol was carried out to confirm the presence of bioethanol in each culture medium. (Gupta et al., 2012)

Test for Bioethanol production (qualitative analysis)

Iodoform test

2 mL of culture broth and 1% Iodine solution was taken in a test tube. In this, dil. Sodium hydroxide was added until the brown colour of the Iodine was discharged. The test tube was gently warmed in a water bath. The yellow precipitate indicated the presence of ethanol.

Potassium dichromate test

2 ml of culture broth was added to a test tube containing Potassium dichromate solution acidified with dilute sulphuric acid. The test tube was gently warmed in a water bath. The change of orange coloured solution to blue indicated the presence of ethanol.

Esterification test

2 ml of culture broth and 1 ml of glacial acetic acid was added followed by the addition of two to three drops of conc. Sulphuric acid. The resulting mixture was then heated in a water bath for 10 minutes. The Fruity smell indicated the presence of ethanol.

Degradation of Paper and Textile waste

The selected bacterial colonies showing greater zone of clearance on CMC plates were isolated and subjected to waste paper and textile degradation to confirm cellulolytic effect. The isolates were separately cultured in basal salt medium containing waste paper and muslin cloth.

10 mL of sterile basal salt media was added to 20 mL test tubes. The media was inoculated with 24hr culture of the selected isolates.

0.2 g of waste paper was added in each test tube and the test tubes were played in a shaker incubator at 45℃ for 10 days. Similarly, 0.2 g of waste paper was added in each test tube and the test tubes were played in a shaker incubator at 45℃ for 10 days. This was done to observe decomposition of paper and muslin cloth in the medium by cellulolytic bacteria. (Guder and Krishna, 2019) with further modification.

Results and Discussion

Isolation and Detection of Cellulose-Degrading Bacteria

Cellulose degrading bacteria were enriched and isolated by spread plate method. A total of 16 isolates were isolated on the basis of the cultural and morphological colony characteristic formed on CMC agar plates. All the bacterial isolates showed proper growth forming mostly white, off-white and mucoid colonies.

The hydrolysis efficiency percentage of the 16 isolates was 60-80% for four isolates, 40-60% for four isolates and 40% and below for eight isolates with five having 0%efficiency. A total of four bacterial isolates were found to be highly positive on screening producing clear zone during aerobic incubation which were as follows: C4 from Compost soil sample, T10 from decaying wood sample and V12, V15 from decaying vegetable sample. The result showed that the Z/N ratio value ranged to between 1.75 to 3.0.

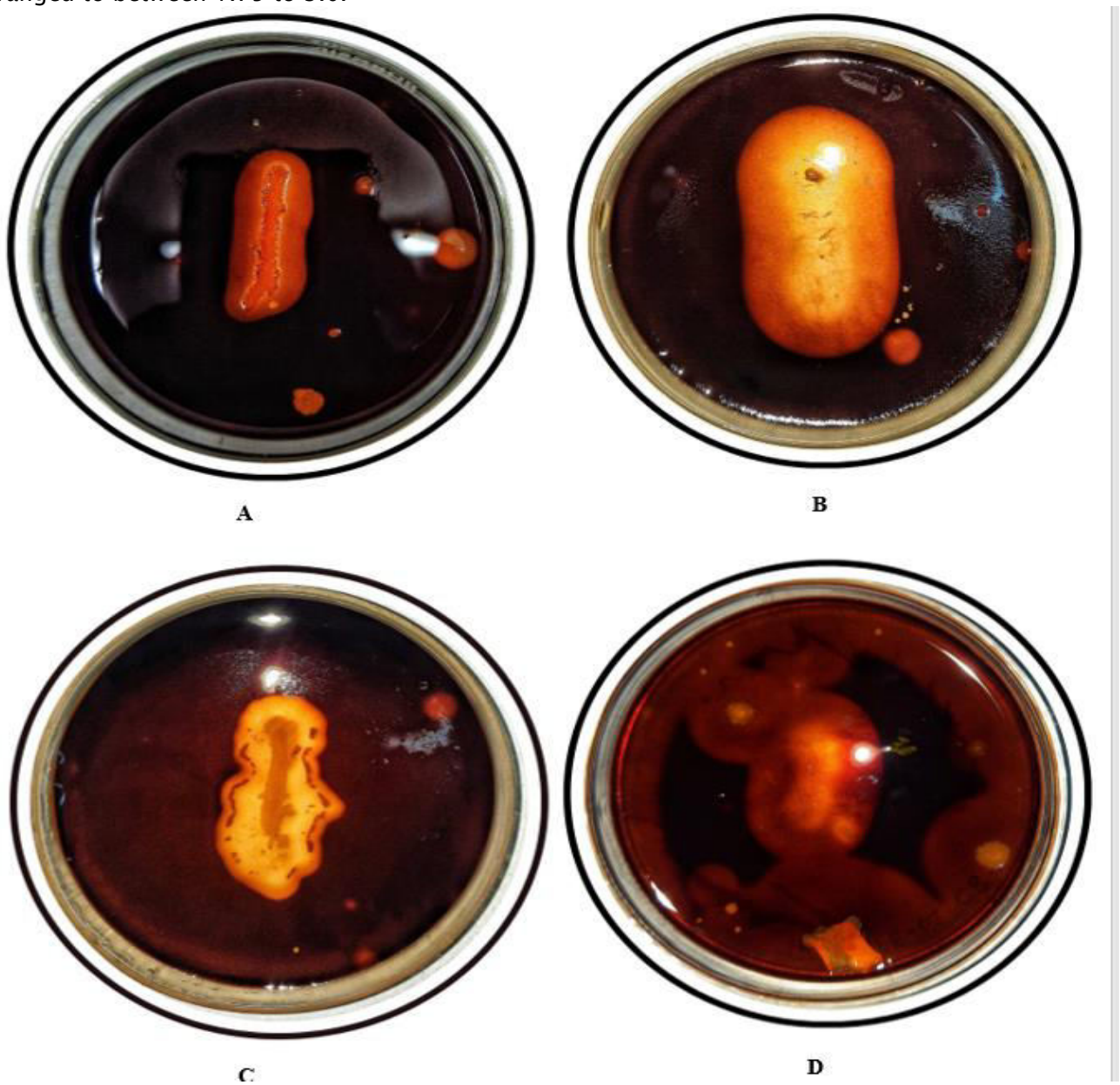

Fig. 1. Illustration of cellulose activity with Gram's iodine solution. Zone of CMC hydrolysis of different isolates A-C4, B- T10, C-V12 and D-V15 showed largest clear zone.

Table 1: Percentage of Efficiency of different Bacterial Isolates based on Starch Hydrolysis Test

Isolate	Total Diameter (cm) (Z+N)	Colony Diameter (N) (cm)	Zone of (Z) hydrolysis (cm)	Z/N ratio	Efficiency Percentage
C1	4.0	2.6	1.4	1.85	35%
C2	1.0	1.0	0	0	0%

C3	6.0	3.0	3.0	1.0	50%
C4	4.0	1.2	2.8	2.3	70%
C5	0.8	0.8	0	0	0%
C6	2.5	1.0	1.5	1.5	60%
C7	3.5	3.0	0.5	0.16	14.28%
C8	1.8	0.8	1.0	1.25	55.55%
C9	4.5	2.4	2.1	0.87	46.6%
T10	1.4	0.4	1.0	2.5	71.42%
T11	2.3	2.3	0	0	0%
V12	2.0	0.5	1.5	3.0	75%
F13	0.6	0.6	0	0	0%
F14	0.4	0.4	0	0	0%
V15	1.1	0.4	0.7	1.75	63.6%
V16	3.4	3.0	0.4	0.13	11.76%

Biochemical and morphological characterization of selected cellulolytic isolates

The bacterial isolates showing highest cellulase production efficiency were selected for characterization on the basis of morphological and biochemical tests. The factors analysed were Colony morphology, Gram's reaction, motility, Carbohydrate fermentation, Catalase production, Oxidase test, Citrate utilisation test, Methyl-red test, Voges-Proskauer test, Indole test, Starch hydrolysis and H_2S production test done on the basis of cultural, biochemical and morphological characterization. The isolates have been identified as **Pseudomonas** sp. (C4), **Micrococcus** sp. (T10), **Bacillus** sp. (V12) and **Staphylococcus** sp. (V15).

Table 2: Morphological and cultural characteristics of the selected bacterial isolates on carboxymethyl cellulose agar plates.

Isolates	Colony color	Margin	Elevation	Opacity	Texture	Gram Staining	Shape under microscope
C4	Mucoid	Regular	Convex	Translucent	Smooth	Gram -ve	Coccus
T10	Yellow	Regular	Convex	Opaque	Rough	Gram +ve	Coccus
V12	White	Regular	Convex	Opaque	Smooth	Gram +ve	Short rods
V15	Off - white	Irregular	Convex	Translucent	Smooth	Gram +ve	Coccus

Table 3: Biochemical test results of selected bacterial isolates

Isolates	C4	T10	V12	V15
Catalase Test	+ve	+ve	+ve	+ve
Voges - Proskauer Test	+ve	-ve	+ve	+ve
Methyl Red Test	-ve	-ve	+ve	+ve
Citrate Utilization Test	+ve	+ve	+ve	-ve
Motility Test	+ve	-ve	-ve	+ve
Sulphide Production Test	+ve	-ve	-ve	-ve
Oxidase Test	+ve	-ve	+ve	+ve
Indole Test	-ve	-ve	+ve	+ve

Starch Hydrolysis Test	+ve	+ve	+ve	+ve
Glucose Fermentation Test	+ve	+ve	+ve	+ve
Gas Production Test during Glucose Fermentation	+ve	-ve	-ve	+ve
Lactose Fermentation Test	+ve	-ve	-ve	+ve
Gas Production Test during Lactose Fermentation	+ve	-ve	-ve	+ve
Sucrose Fermentation Test	+ve	-ve	-ve	+ve
Gas Production Test during Sucrose Fermentation	+ve	-ve	-ve	+ve

Enzyme activity assay

The extracted cellulase enzyme underwent analysis using the DNS (3,5-dinitrosalicylic acid) method to quantify the quantity of reducing sugars released from the substrate. Enzyme activity was characterized by the quantity of glucose (reducing sugar) generated from CMC (Carboxymethyl cellulose) within one minute under standard conditions.

Based on the enzyme activity assay V15 showed greater activity with a value of 0.1473U/ml and T10 showed lowest activity with a value of 0.1259U/ml.

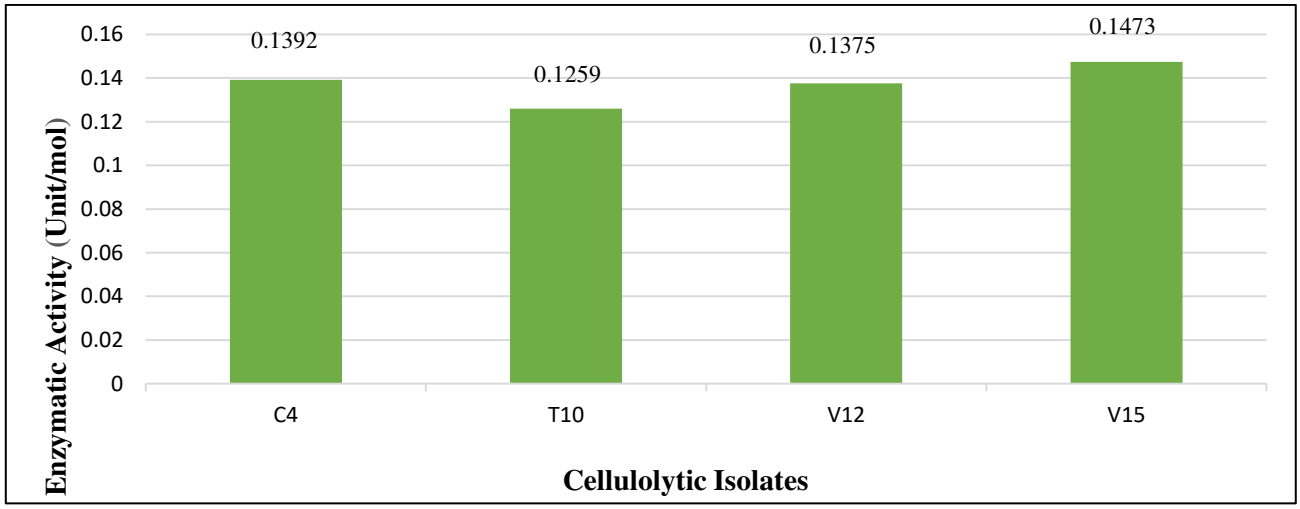

Fig: 3. Graph showing enzymatic activity of selected bacterial isolates where V15 had the highest activity followed by C4, V12 and least activity of T10.

Bioethanol formation

The experimental setup involved the simultaneous fermentation and saccharification of bacterial cultures (C4, T10, V12, and V15) with Saccharomyces cerevisiae, resulting in ethanol production. The experiment indicated the high cellulolytic potential of these selected bacterial isolates in decomposition of cellulose, a complex polysaccharide and its assistance in high rate fermentation for ethanol production. Co-culturing of bacterial strains with yeast sp. and simultaneous Saccharification and fermentation of ethanol was reported by several workers. (Lenziou et al., 1994) and (Eklund and Zacchi, 1995)

Results indicated that momentous synergistic cellulose degradation can be achieved in the mixed culture system of cellulolytic bacteria and non-cellulolytic yeast in which non cellulolytic yeast Saccharomyces cerevisae utilises the broken sugar derived from cellulose degradation and converts

it to ethanol. The result signifies the importance of cellulolytic bacteria in conversion of complex polysaccharide into innovatory green fuel like bioethanol.

The bacterial isolates T10, V12 and V15 showed high potential to convert cellulose into reducing sugars which could be readily used in many applications like feed stock for production of organic compounds that have high industrial value; for example, in the contemporary study this has been demonstrated by concurrent Saccharification and fermentation of cellulose into ethanol. (Gupta et al., 2012)

Table 4: Qualitative Analysis test for Bioethanol Production

Sample	Iodoform Test	Esterification Test	Potassium Dichromate Test
Basal Media	-ve	-ve	-ve
Basal Media with culture	-ve	-ve	-ve
Basal Media with Yeast	+ve	+ve	Slightly positive
C4	+ve	+ve	Slightly positive
T10	+ve	+ve	+ve
V12	+ve	+ve	+ve
V15	+ve	+ve	Slightly positive

Paper and Textile degradation test

Among the four designated isolates, two isolates (C4 and V12) were observed capable of waste paper degradation which was seen as decomposed waste paper forming a turbid solution in the test tube. One isolate V15 showed slight degradation whereas almost no degradation was observed in the T10 test tube. In context of textile degradation, two isolates were able to degrade muslin cloth forming a turbid solution. Maximum textile degradation was accounted for the isolate C4 whereas the isolate V15 accounted for slight degradation of the textile.

The result of waste paper and textile degradation indicated that not all bacterial isolates which show hydrolytic effects on CMC are able to digest cellulose in waste paper and muslin cloth. Hence, bacterial isolate which can degrade waste paper and muslin cloth were considered to have more cellulolytic effect. The following experiment open routes involving the proper recycling of paper and textile waste leading to prominent minimization of waste when applied on an industrial scale.

Diameter of clear zone on CMC agar ranged from 4.0 cm to 1.1 cm. The highest diameter of the clear zone was seen in C4 and the lowest clear zone diameter was seen in V15. The highest hydrolytic value was seen in V12 and the lowest value was seen in V15. The current study shows that there is a positive correlation between hydrolytic value and waste paper degradation effect of the isolates in isolates with higher hydrolytic value on CMC waste paper degradation affect is positive and vice versa.

Table 5: Hydrolytic value of selected Bacterial Isolates and Paper and Textile Degradation Analysis

Isolates	Diameter of Clear Zone	Colony Diameter	Hydrolytic Value	Paper Degradation	Textile Degradation

	(cm)	(cm)			
C4	4.0	1.2	3.30	++	++
T10	1.4	0.4	3.50	–	–
V12	2.0	0.5	4.00	++	–
V15	1.1	0.4	2.75	+	+

Conclusion

The major aim of the present work was to carry out isolation and identification of capable cellulase producing bacterial strains from diverse sources such as decaying wood, compost soil, decaying vegetables and fruits. The rare microbial communities found in these sources provide a rich pool of Cellulolytic potential, essential for breakdown of cellulose rich substrates. Four isolates were obtained from decaying wood, compost soil, decaying vegetables each of them characterized for morphological and biochemical analysis. These isolates were found to be Pseudomonas, Bacillus, Micrococcus and Staphylococcus. Partial Purification of enzymes was done and the enzymatic activity was determined by the DNS method. Through meticulous research and innovation, scientists have harnessed these microbial Marvels, positioning these as pivotal players in the transition towards eco-friendly energy solutions. Green fuel indulging bioethanol production capability was examined by simultaneous saccharification by the divergent isolates and fermentation by Saccharomyces cerevisae.

In conclusion, the Cellulolytic Crusaders presents a promising avenue in breaking down cellulose-rich materials, transforming then into valuable biofuels while simultaneously addressing environmental challenges. Consequently, the cellulase enzyme produced is examined for biodegradation of cellulosic materials such as waste paper and muslin cloth which showed that their unique ability to breakdown cellulose not only offers a renewable source of energy but also addresses environmental challenges by reducing waste accumulation.

As we navigate the challenges of a rapidly changing global landscape, investing in and further understanding these cellulolytic organisms remains imperative. By doing so we can unlock unprecedented avenues for environmental stewardship, economic growth and a greener future for generations to come.

Acknowledgement: The authors express their sincere thanks to the Principal, Patna Women's College (Autonomous), Patna University for providing the grant and infrastructural facility for the successful completion of this research work

References

1. Devi M.H. and Munjam, S. (2022) Bioethanol production from rice straw and cellulose degradation using Aspergillus terreus and Trichoderma harzanium, Biosci. Biotechnol. Res. Asia, vol. 19, pp. 699-711.
2. Dsouza, R. and Chandak, Dr. N. (2018) Isolation and identification of cellulose degrading bacteria from grave yard soil and termite tubes and its application in ethanol production, J. Emerg. Technol. Innov. Res., vol. 5, pp. 632-646.
3. Eklund, R. and Zacchi, G. (1995) Simultaneous saccharification and fermentation of stream-pretreated willow. Enzyme Microb. Technol., vol. 17, pp. 255-259.
4. Guder, D.G. and Krishna, M.S.R. (2019) Isolation and characterization of potential cellulose degrading bacteria from sheep rumen, J Pure Appl Microbiol, vol. 13, pp. 1831-1839.

5. Gupta, P., Samant, K., Sahu, A. (2012) Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential, Int J Microbiol, pp. 1-5.
6. Islam, F. and Roy, N. (2018) Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses, BMC Res. Notes, vol. 11, pp. 1-6.
7. Khianngam, S., Pootaeng-on Y., Techakriengkrai T., Tanasupawat S. (2014) Screening and identification of cellulase producing bacteria isolated from oil palm meal, J. Appl. Pharm. Sci., vol. 4, pp. 090-096.
8. Korsa, G., Konwarh, R., Masi, C., Ayele, A., Haile, S. (2023) Microbial cellulase production and its potential application for Textile Industries, Ann. Microbiol, (73), pp. 1-21.
9. Lenziou, V., Christakopoulos, P., Kekos, D., Macris, B. J. (1994) Simultaneous saccharification and fermentation of sweet sorghum carbohydrates to ethanol in a fed batch process. Biotechnol. Lett., vol. 16, pp. 983-988.
10. Sethi, S., Datta A, Gupta BL, Gupta S. (2013) Optimization of cellulase production from bacteria isolated from soil, ISRN Biotechnol., vol. 1, pp. 1-7.
11. Singh, S., Singh, V. K., Aamir, M., Dubey, M. K., Patel, J. S., Upadhyay, R. S., Gupta, V. K. (2016) Cellulase in pulp and Paper Industry, New and Future Developments in Microbial Biotechnology and Bioengineering, vol. 1, pp. 152-162.
12. Sulyman, A.O., Igunnu, A., Malomo, S.O. (2020) Isolation, purification and characterization of cellulase produced by aspergillus niger cultured on arachis hypogaea shells, Heliyon, vol. 6, no. 12, pp. 1-10.
13. Thomas, A., Laxmi, M., Benny, A. (2021) Bioethanol production of cellulase producing bacteria from soils of Agrowaste Field, J. Sci. Res., vol. 13, pp. 643-655.