The Pharma Innovation Journal 2022; 11(12): 2500-2507

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 2500-2507

www.thepharmajournal.com
Received: 29-10-2022
Accepted: 30-11-2022

BP Bahatkar
Department of Botany,
Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola,
Maharashtra, India

SJ Gahukar
Department of Botany,
Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola,
Maharashtra, India

AA Akhare
Department of Botany,
Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola,
Maharashtra, India

DR Rathod
Department of Botany,
Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola,
Maharashtra, India

AM Charpe
Department of Plant Pathology,
Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola,
Maharashtra, India

YV Ingle
Department of Plant Pathology,
Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola,
Maharashtra, India

Corresponding Author:
BP Bahatkar
Department of Botany,
Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola,
Maharashtra, India

Isolation, screening and identification of cellulose-degrading bacteria from different types of samples

BP Bahatkar, SJ Gahukar, AA Akhare, DR Rathod, AM Charpe and YV Ingle

Abstract
Microbial utilization of cellulose is the key factor for the utmost material flow in the biosphere. Despite this vast number of cellulase producers, there is a deficiency of microorganisms that can produce significant amount of the cellulase enzyme to efficiently degrade cellulose to fermentable products. Little emphasis has been given to cellulase production from bacteria despite their extremely high natural diversity, which endows them with the capability to produce stable enzymes. The present study aimed at the isolation, screening and identification of cellulose degrading bacteria isolated from different samples like soil, wood logs, forest liters and forest. Total 20 bacterial cultures were isolated through serial dilutions and spread plate method in carboxymethyl cellulose (CMC) agar media. To indicate the cellulase activity of the bacterial isolates, diameter of clear zone around the colony and hydrolytic value on Congo red agar media were measured. CDB 12 and CDB 09 exhibited the maximum zone of clearance around the colony with the hydrolytic value of 4.4 and 3.75 respectively. The cultures were also further tested for their capacity to degrade filter paper by using filter paper strip method. The maximum filter paper degradation percentage was estimated to be 21.33% for CDB 12. Total 9 isolates were selected on the basis of primary and secondary screening, which were then identified morphologically and biochemically by performing different biochemical tests and Gram staining test.

Keywords: Cellulose degrading bacteria, CMC agar, cellulase, cellulolytic activity, morphological analysis and biochemical analysis

Introduction
Cellulose is a major component of plant biomass. It is an abundant, cheap biopolymer, and a renewable resource of energy (Khaleel *et al*., 2018) [21]. Approximately 100 billion metric tons cellulose is produced naturally every year, while the entire biomass is around 280 billion metric tons (Cheng *et al*., 2010) [11]. Cellulose is mainly a polysaccharide having a fibrous crystalline appearance and made up of the repeating units of D-glucose, which is linked by β-1, 4- glycosidic linkage (Zaghoud, *et al*., 2019) [38]. It can be changed into glucose and other soluble sugars by the process called cellulolysis. For cellulolysis, a set of enzymes named cellulase are required, which includes endoglucanase (endo-1, 4-β-D-glucanase); cellobiohydrolase or exoglucanase (exo-1, 4-β-Dglucanase), and β-glucosidase (1, 4-β-D-glucosidase) (Li and Gao, 2008) [23]. Different microorganisms produce this inducible bioactive compound during their growth and development on cellulosic matters (Gomashe *et al*., 2013) [13]. Cellulolytic microorganisms mostly fungi and bacteria are involved in cellulosic compound degradation in soils (Ojumu *et al*., 2003) [28]. However, there is a deficiency of microorganisms that can produce a significant amount of cellulase enzyme to efficiently convert cellulose into fermentable products (Maki *et al*., 2009) [25]. Bacteria are now being widely explored for cellulase production because of their extremely high natural diversity and the capability to produce stable enzymes that can be applied in industries (Haakana *et al*., 2004; Ashjaran and Sheybani, 2019) [15, 2].
A great number of researches have been conducted to develop efficient means to convert biomass to high value products [2-5]. However, the recalcitrant nature of lignocellulosic biomass has presented a major obstacle for conversion and utilization of cellulosic biomass. Although different physical and chemical methods for breaking down cellulosic substances have been established to effectively hydrolyze complex cellulosic polymers, physicochemical treatments often require harsh and extreme conditions [6]. Biological treatments are, thus, considered a promising approach for utilizing cellulosic waste because most of the isolated microbial enzymes can often catalyze hydrolytic reactions under ambient environment.

To overcome these problems, microbial composting is the upcoming technologies for cellulosic waste disposal in which biodegradation of waste is carried out using efficient microbial communities of lignocellulolytic microorganisms which can degrade lignocellulosic biomass within short period of time. Bacteria, actinomycetes, fungi, algae and protozoa are the major microorganisms found in soil which decompose soil organic materials, of which bacteria and fungi are prominent and abundant ones. Microbes use the waste for their own metabolism and finally produce some simple and useful compounds which are important for soil health, plant growth and overall eco-balance.

Material and Methods

Sample collection

Samples were collected from Melghat Forest region and were stored at 4 °C in sterile containers until inoculation. Tenfold serial dilutions of each sample were prepared in sterilized distilled water and 0.1 ml of that diluted sample was spread on Carboxymethyl cellulose medium for isolation. (Dhingra and Sinclair, 1993) [12].

Isolation and Purification cellulolytic bacteria

Cellulolytic bacterial strains were isolated from various samples by Dilution plate technique. Serial dilutions was done by weighing 1gm of sample in 9ml of distill water in a test tube (1: 10). After that 1ml of suspension was transferred from first test tube to second test tube containing 9 ml of sterile distilled water (1: 100) from second test tube to third test tube containing 9 ml of sterile distilled water (1: 1000). Similar dilution process was continued as per requirement. Bacterial culture was inoculated in CMC (Carboxy-methyl cellulose) medium [1] supplemented with 1% CMC (Hi Media) and incubated at 300 C for 24 hours.

Determination of cellulase producing activity of the bacterial isolates

The medium used for determination of cellulase producing activity of the bacterial isolates was carboxymethyl cellulose agar (CMC agar) with the following composition (g/l): peptone 10.0, carboxymethyl cellulose (CMC) 10.0, K2HPO4 2.0, MgSO4.7H2O 0.3, (NH4)2SO42.5, gelatin 2.0 and agar 15, pH was adjusted at 6.8-7.2, and the plates were incubated at 35 °C for 24hours. After incubation for 24 hours, CMC agar plates were flooded with 0.1- 0.2% Congo red and allowed to stand for 15 min at room temperature. 1M NaCl was thoroughly used for counterstaining the plates. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis. The bacterial colonies having the clear zone were identified as cellulose degrading and selected for further studies.

Screening of bacterial cultures

- Primary preliminary screening of different microbial cultures was carried out by estimation of cellulase activity of isolated cultures in CMC agar plate through halo zone formation

Pure cultures of bacterial isolates were individually transferred on CMC agar plates. After incubation for 15 days, CMC agar plates was flooded with 1% Congo red and allowed to stand for 15 min at room temperature. One molar NaCl was used for counterstaining the plates. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis. The diameter of the bacterial colony (CD) and clear zone (ZD) around the colony after Congo red and NaCl treatment was measured. The ratio (R) of ZD and CD was also calculated to identify the highest cellulase activity of bacterial isolates. The largest ratios were assumed to have the maximum cellulase producing activity. The bacterial colonies having the largest clear zone were selected for further analysis.

- Secondary preliminary screening of different microbial cultures was carried out by adopting filter paper strip method described by Somani and Wangikar (1979) [34-35].

The sterilized petri plates were poured with 2 percent plane agar medium. Two sterilized filter paper strips of 7 x 1 cm were placed parallel at 3 cm apart on the surface of the medium in each plate. Spore suspension of each culture of bacteria in distilled sterilized water was poured on the centre of the strips and on the medium in between the strips. Each culture was replicated three times then; the plates were incubated for 15 days at 28°±2 °C. The growth on the strips and on the medium was compared and also loss in weight of filter paper strips was estimated. The cultures which grow luxuriantly and sporulated profusely on filter paper strips and recorded maximum loss in weight of filter paper strips were designated as cellulolytic cultures.

Biochemical tests were performed to characterize all the isolates as per standardized tests (Shinde, 2017) [33].

Oxidase test

A small piece of filter paper was soaked in 1% Kovac‘s oxidase reagent and let dry. A loop was used to pick a well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate and was rubbed onto treated filter paper and observed for color changes. Microorganisms were oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms were delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms were oxidase negative if the color does not change or it takes longer than 2 minutes.

Catalase test

This test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh cultures of pure isolates were taken on glass slides and one drop of H2O2 (30%) was added. Appearance of gas bubble indicated the presence of catalase enzyme.

Indole test

Indole test was used to determine the ability of an organism to spilt amino acid tryptophan to form the compound indole.

Methods

a) The tryptophan broth was inoculated with the broth culture or emulsified isolated colony of the test organism in tryptophan broth.
b) Incubated at 37 °C for 24-28 hours in ambient air.
c) 0.5 ml of Kovac‘s reagent was added to the broth culture.

Expected results: Positive: Pink colored rink after addition of appropriate reagent

Negative: No color change even after the addition of appropriate reagent.

MR-VP test

Sterilized glucose- phosphate broth tubes were inoculated with the test culture and incubated at 28±2 °C for 48 h. After incubation five drops of methyl red indicator were added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production was taken as negative for the test.

Casein hydrolysis test / skimmed milk agar utilization

Milk agar contains skim milk (lactose and casein), peptone, and agar. Many organisms can grow on this medium. This medium is used to detect the production of proteases/caseases that digest casein to soluble peptides. This results in a clear zone. Soluble peptides can then be absorbed by the cell. Casein is responsible for the white color of milk. When digested by exoenzymes, the white agar turns clear and colorless. Bacterial pigments can be seen distinctly on this agar.

Citrate utilization test

Isolates were streaked on Simmon's citrate agar plate and incubated at 28±2 ºC for 24 hr. Change in colour from green to blue indicated the positive reaction for citrate utilization whereas lack of growth and no change in colour indicated negative reaction.

Lignin media utilization test

Microbial cultures were screened for lignolytic activity. The preferential degradation of lignin component was determined by the method described by Ander and Erikson, 1977 [4]. In which radial growth of microbes were recorded and observed after incubation for brown colour formation on back side of plates. This indicated the presence of lignolytic enzymes.

Results and Discussion

Collection and isolation of sample

Sixteen different samples were collected from different places of Melghat forest region. Twenty different bacterial cultures were isolated from sixteen different collected samples by dilution plate technique and pure bacterial colonies were obtained by repeated streaking on CMC media. (Pointing, 1999 and Shinde, 2017) [39, 33]

Generally microbial decomposer are carrier based ready to use live fungal or bacterial formulation, which on application to composting pits or substrates helps in mobilization of various nutrients by their biological activities. The product may be in liquid or carrier based formulation capable of holding very high population of specific microorganisms and it should be free from other contaminating microorganism. Isolation and identification of appropriate strain of a decomposer is a foremost important. Keeping in view twenty microbes were isolated from various sources. Organic material like forest litters, forest waste including infected plant, soil, wood logs and water samples were collected for obtaining efficient strains.

Preliminary screening of different microbial cultures

Primary preliminary screening of different microbial cultures were carried out by estimation of cellulase activity of isolated cultures in CMC agar plate through halo zone formation

The 20 cultures isolated from various different samples were tested for their cellulolytic activities by culturing pure cultures of bacterial isolates on CMC agar plates. The experiment was performed in three replications and after 15 days of incubation, cultures showing clear zones around growing bacterial colonies were considered as cellulolytic indicating cellulose hydrolysis. The bacterial colonies having the largest clear zone with HC value more than 2 were selected for further analysis. (Pointing, 1999) [39] The details are given in Table 1, Plate 1. and Fig.1.

All the cultures were found at par however, CDB 12 found significantly superior over others and recorded maximum HC value (4.4) followed by CDB9 (3.75), CDB5 (3.4), CDB15 (03) and CDB10 (2.8). Total 14 isolates were selected for further screening as they have shown HC value more than 2.

Several lines of evidence also supports the present observations that cellulose degrading bacteria from different samples like soil, agro waste etc., were isolated and screened on the basis of Congo red staining.

Ponnambalam *et al*. (2011) [31] isolated cellulose degrading bacteria from various natural environments. Six bacterial isolates were isolated and comparatively analysed for effective production of cellulase enzyme. Among the six bacterial isolates, a bacterium F was found to be effective producer. It has the shown the clearing zone of 1.9 cm compared to next effective producer having clearing zone of 1.7 cm.

Pratima *et al*. (2012) [32] isolated the cellulose degrading bacteria (CDB) by enriching the basal culture medium with filter paper as substrate for cellulose degradation. To indicate the cellulose activity of the organisms, diameter of clear zone around the colony and hydrolytic value on cellulose Congo red agar media were measured. CDB-8 and CDB-10 exhibited the maximum zone of clearance around the colony with diameter of 45 and 50 mm and with the hydrolytic value of 9.0 and 9.8, respectively.

Behera *et al*. (2014) [4] isolated cellulose degrading bacteria from mangrove soil of Mahanadi river delta, Odisha, India. Results showed that total fifteen cellulose degrading bacteria were isolated based on their halo zone formation on Congo red agar medium. Their maximum CMC hydrolysis capacities (HC value) ranged from 1.18 to 2.5 cm.

Abedin (2015) [1] isolated cellulose degrading bacteria from soil samples collected from National parliament area and BRAC nursery. The five isolates were screened for cellulolytic activity using Congo red stain on Carboxymethyl cellulose (CMC) agar plates among which CBD - 3, CDB - 4 and CDB-5 showed largest clear zone and HC value i.e. 2.4 mm, 3.6 mm and 2.0 mm.

Secondary preliminary screening of different microbial cultures was carried out by adopting filter paper strip method described by (34)

The 14 bacterial isolates selected on the basis of primary preliminary screening were tested for their cellulolytic activities by using filter paper strips method. The bacterial culture which grew luxuriantly, profusely and showed maximum percent loss in weight of filter paper strips were considered as cellulolytic. The details are given in Table 2, Plate 2 and Fig.2.

All the cultures are found at par however, CDB 12 found significantly superior over others and recorded maximum loss in weight of filter paper strips (21.33%) followed by CDB5 (19.46%), CDB15 (19.13%), CDB16 (18.93%) and CDB19 (18.82%).

Filter paper strips method (Somani and Wangikar, 1979) [34-35] was used in preliminary screening of the isolates. It was noticed that most of the cultures grew well on strips rather than on media and had shown luxuriant growth and sporulation on filter paper strips indicating that these bacteria can utilize filter paper as sole carbon source.

Present findings agree with those of Bhagat (2008) [5] and Pande (1978) [30] that the bacterial strains could use Whatman filter paper as a sole carbon source and the utilization of filter paper for comparing the growth of several isolates on strips and on medium.

Bhagat (2008) [5] carried out preliminary screening of different fungal and bacterial cultures by adopting filter paper strip method and reported that among bacterial cultures, C. bibula had shown maximum weight loss (17.140/0) followed by *B. polmyxa* (15.24%), P. striata (13.340/0) while Lactobacillus sp. recorded minimum loss i.e. 12.85 percent of filter paper strips.

Total 9 isolates were selected for further screening on the basis of the results of secondary preliminary screening.

Characterization of bacteria isolates

Morphological characterization

The bacterial isolates were presumptively characterized by means of morphological characteristics which were determined by Gram stain test. The details are given in Table 3.

All the isolates retained the purple color of crystal violet stain implying that they were Gram positive rod shaped.

Similar work was carried out by Abedin (2015) [1] where morphological features were determined by Gram stain test and all the all three isolates showed positivity in Gram staining test indicating that they were Gram positive organisms.

Kumar (2016) [20] performed morphological characterization of 70 isolates by gram staining test and found all the isolates were gram negative rod and cocci shaped.

Biochemical characterization

After the morphological characterization, all the nine isolates which were selected on the basis of results of preliminary screening were tested for different biochemical characters by performing various biochemical tests as per standardized tests (Shinde, 2017) [33]. Some of the tests were performed for comparing the characteristics depicted in safrinet manual for phyto bacteriology and Bergey's Manual of Determinative Bacteriology (Krieg and Dobereiner, 1984) [22]. The results of which are given below. (Table. 4 and Plate.3 – Plate.7)

The biochemical test were conducted for all 9 bacterial isolates of which 7 isolates showed positive catalase activity, 2 isolates was positive for oxidase activity, 7 isolates were positive for indole reaction, 5 isolates were positive for MR-VP test, 5 isolates were positive for citrate utilization, all were positive for skimmed milk agar test/ casein hydrolysis test and all were positive for LM utilization. Morphological and biochemical assays suggested that the cellulose-degrading bacterial isolates are members of the genus *Bacillus sp* and *Streptomyces sp.*

Kumar (2016) [20] conducted biochemical test for 70 bacterial isolates and found out of 70 isolates, 27 isolates were positive for indole reaction, 60 isolates positive catalase activity, all isolates were positive for oxidase activity, 61 isolates were positive for gelatine liquefaction, 31 isolates were positive for Voges Proskauer test and 57 isolates were positive for citrate utilization.

Abedin (2015) [1] performed biochemical test for 3 bacterial isolates and found out of 3isolates, 2 isolates were positive for MR-VP test, all the three isolates were negative for oxidase and indole test and positive for catalase, citrate utilization and casein hydrolysis test.

Table 1: Estimation of cellulase activity of cellulose degrading bacteria in CMC agar plate through halo zone formation

Sr. No.	Isolate No	Zone Diameter (ZD) (cm)	Colony Diameter (CD) (cm)	HC Value (ZD/CD)
1	CDB 1	4.5	2.4	1.87
2	CDB 2	1.3	0.5	2.60
3	CDB 3	2.1	1.0	2.10
4	CDB 4	2.0	0.9	2.20
5	CDB 5	1.7	0.5	3.40
6	CDB 6	1.2	0.7	1.70
7	CDB 7	1.3	0.6	2.20
8	CDB 8	1.1	0.4	2.40
9	CDB 9	1.5	0.4	3.75
10	CDB 10	1.4	0.5	2.80
11	CDB 11	1.2	0.8	1.50
12	CDB 12	4.0	0.9	4.40
13	CDB 13	1.0	0.6	1.66
14	CDB 14	3.5	1.3	2.69
15	CDB 15	3.0	1.0	3.00
16	CDB 16	2.6	1.0	2.60
17	CDB 17	0.9	0.5	1.80
18	CDB 18	1.6	1.0	1.60
19	CDB 19	1.5	0.6	2.50
20	CDB 20	2.0	0.9	2.20
	F test	Sig.	Sig.	
	SE(m)±	0.070	0.030	
	C.D.	0.200	0.087	

Table 2: Percent loss in weight of filter paper strip after 15 days of incubation

Sr. No.	Isolates	Mean of weight filter paper after 15 DOI (mg)	Weight Loss of filter paper strip (mg)	% Loss in weight of filter paper strip
	Control	150.0	00.0	00.0
	CDB 2	121.9	28.1	18.73 (25.63)
	CDB 3	124.1	25.9	17.26 (24.54)
	CDB 4	123.5	26.5	17.66 (24.84)
	CDB 5	120.8	29.2	19.46 (26.17)
	CDB 7	123.2	26.8	17.86 (24.99)
	CDB 8	123.5	26.5	17.66 (24.84)
	CDB 9	119.9	24.8	16.55 (26.60)
	CDB 10	122.0	28.0	18.66 (25.58)
	CDB 12	118.0	32.0	21.33 (27.49)
	CDB 14	122.1	27.9	18.60 (25.53)
	CDB 15	121.3	28.7	19.13 (25.92)
	CDB 16	121.6	28.4	18.93 (25.78)
	CDB 19	121.8	28.2	18.82 (25.70)
	CDB 20	122.0	28.0	18.66 (25.58)
	F test	Sig.		
	SE(m)±	0.374		
	C.D.	1.084		

Figure in parenthesis () Arc sine transformed value
(Initial wt. of filter paper strip = 150 mg)

Table 3: Observations of isolates morphological features

Sr. No.	Isolate No.	Gram +ve/-ve	Cell Shape	Spore Forming/ Non- Spore forming
1	CDB02	+ve	Rod Shaped	Spore Forming
2	CDB05	+ve	Rod Shaped	Spore Forming
3	CDB 10	+ve	Rod Shaped	Spore Forming
4	CDB12	+ve	Rod Shaped	Spore Forming
5	CDB14	+ve	Rod Shaped	Spore Forming
6	CDB15	+ve	Rod Shaped	Spore Forming
7	CDB16	+ve	Rod Shaped	Spore Forming
8	CDB19	+ve	Rod Shaped	Spore Forming
9	CDB20	+ve	Rod Shaped	Spore Forming

Table 4: Observations of Biochemical test

Sr. No.	Biochemical Test	CDB 2	CDB 5	CDB 12	CDB 10	CDB 14	CDB 15	CDB 16	CDB 19	CDB 20
1.	Catalase	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
2.	Oxidase	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
3.	Indole	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
4.	MR- VP	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve
5.	Citrate	+ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve
6.	LM	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
7.	Skim Milk Agar	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

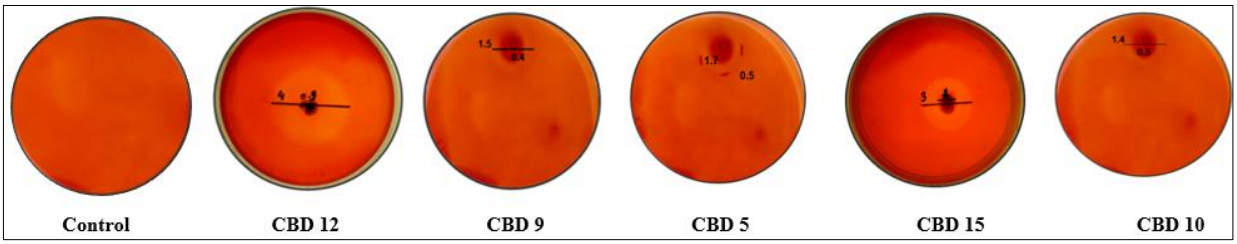

Plate 1: Cellulase activity of cellulose degrading bacteria showing maximum clearing zone and hydrolytic capacity (HC) value on CMC agar plate

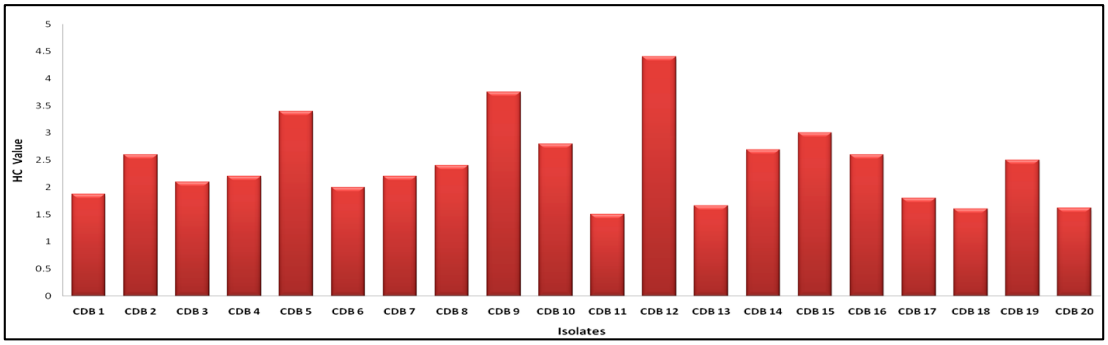

Fig 1: Graphical representation of cellulase activity of cellulose degrading bacteria on CMC agar plate through halo zone formation

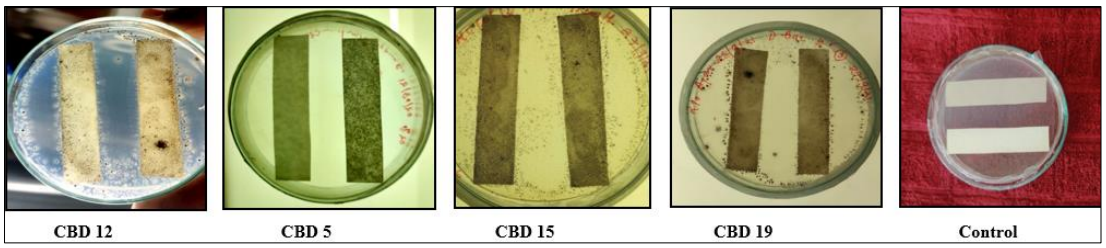

Plate 2: Observations of Filter Paper Strip Method

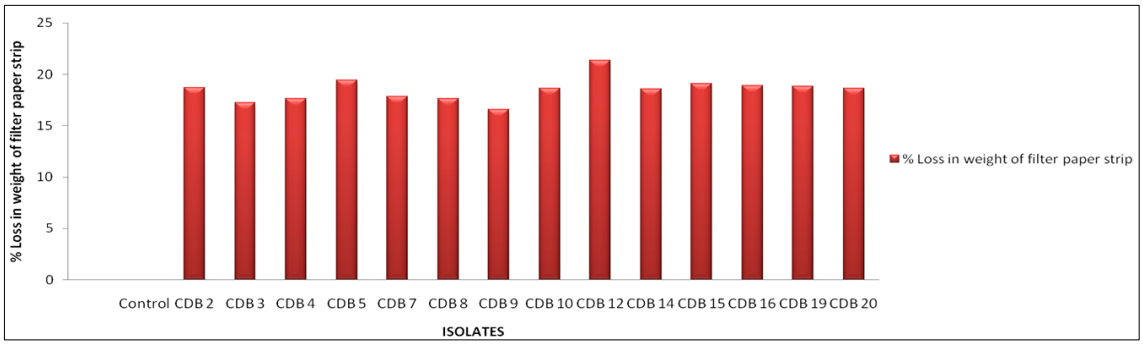

Fig 2: Observations of Filter Paper Strip Method

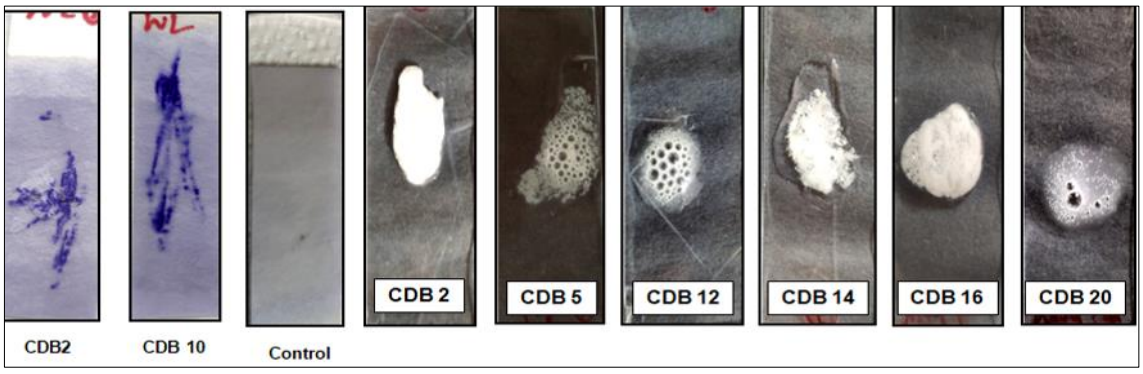

Plate 3: Observations of Oxidase and Catalase Test

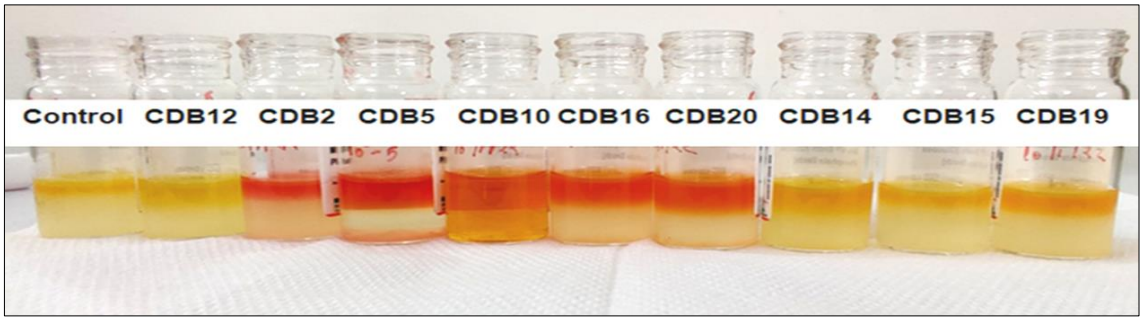

Plate 4: Observations of MR-VP Test

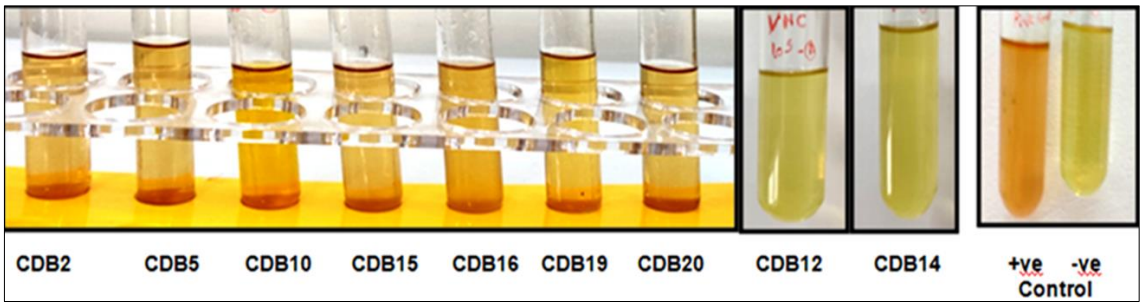

Plate 5: Observations of Indole Test

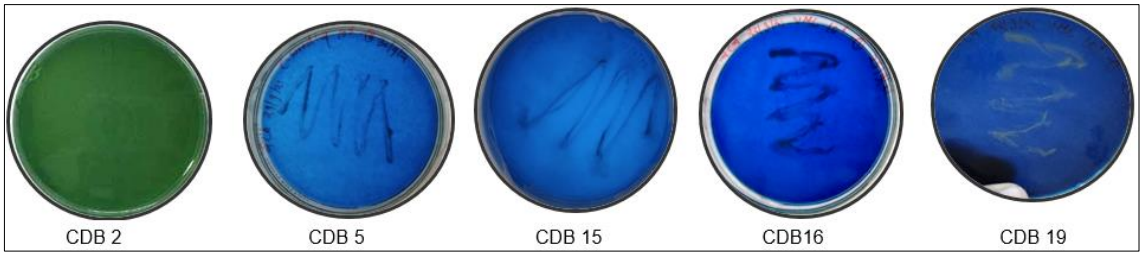

Plate 6: Observations of Citrate Agar Utilization Test

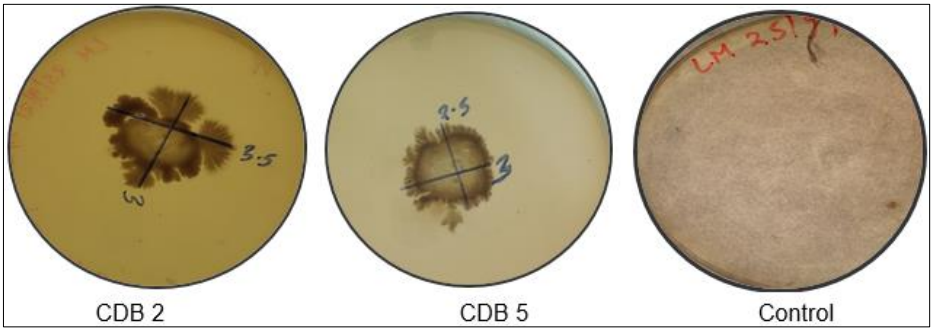

Plate 7: Observations of LM Agar Utilization Test

Conclusion

Among the 20 cultures isolated from different samples, 14 cultures have shown the highest clear zone i.e halo zone formation in CMC agar plate showing their cellulose degrading capacity. Among the 14 isolates, 9 isolates have shown the growth on filter paper strip with maximum degradation i.e having high potential of lignocellulose degradation. These efficient 9 isolates were identified on the basis of morphological and biochemical characterization. These selected potential nine bacterial cultures are capable of lignocellulosic biomass degradation. This study might be potentially useful candidates for efficient cellulosic biomass conversion and can be used as inoculants for microbial composting to enhance the degradation of cellulose of which the agricultural waste is composed of.

References

1. Abedin. Isolation and Identification of Cellulose Degrading Bacteria from Soil Sample; c2015.
2. Ashjaran A, Sheybani S. Drug Release of Bacterial Cellulose as Antibacterial Nano Wound Dressing. International Journal of Pharmaceutical Research and Allied Sciences. 2019;8(3):137-143.
3. Batubara UM, Mardalisa M, Suparjo S, Maritsa HU, Pujianto E, Herlin M. Isolation and Characterization of Cellulolytic Bacteria Diversity in Peat land Ecosystem and Their Cellulolytic Activities, Earth and Environmental Science. 2021;934:012028.
4. Behera BC, Parida S, Dutta SK, Thatoi HN. Isolation and Identification of Cellulose Degrading Bacteria from Mangrove Soil of Mahanadi River Delta and Their Cellulase Production Ability. American Journal of Microbiological Research. 2014;2(1):41-46.
5. Bhagat D. Enrichment of Compost through Microbial Inoculants and Chemical Amendments Ph.D. Thesis (Unpub.) PKV, Akola; c2008.
6. Bhagat SA, Kokitkar SS. Isolation and identification of bacteria with cellulose-degrading potential from soil and optimization of cellulase production, Journal of Applied Biology and Biotechnology. 2021;9(06):154-161.
7. Bharti, Sandhu. Partial Purification of Cellulase Produced By A Bacterium Isolated from Wood Decompost, Int. J Pure App. Biosci. 2015;3(4):208-215
8. Bhowmick, Sengupta. Enumeration of Soil Inhabiting Cellulolytic Bacteria as Plant Growth Promoter International Journal of Life Sciences. 2015;9(6);50-55.
9. Bhuvaneshwari S, Hettiarachchi H, Meegoda JN. Crop Residue Burning in India: Policy Challenges and Potential Solutions, Int. J Environ. Res. Public Health. 2019;16:832.
10. Bremner JM. Total organic carbon in methods of soil analysis Part- 2 chemical and micro-biological properties. Page, A. L. (ed). II Edn. Amer. Soc. Agron. Inc. and Soil Sci. Amer Inc. Madison, Wisconsin, USA; c1970. p. 475-

594.
11. Cheng Q, Wang J, McNeel JF, Jacobson PM. Water Retention Value Measurements of Cellulosic Materials Using a Centrifuge Technique. Bio Resources. 2010;5(3):1945-1954.
12. Dhingra OD, Sinclair JB. Basic plant pathology methods. CBS Publisher New Delhi; c1993. p. 179-180.
13. Gomashe AV, Gulhane PA, Bezalwar PM. Isolation and screening of cellulose degrading microbes from Nagpur region soil. International Journal of Life Science. 2013;1:291-293.
14. Gupta P, Samant K, Sahu A. Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential. International Journal of Microbiology; c2012.
15. Haakana H, Mittinen-Oinonen A, Joutsjoki V, Mantyla A, Souminen P, Vahmaanpera J. Cloning of cellulase from *Melanocarpus albomyces* and their efficient expression in *Trichoderma reesei*. Enzyme Microbial Technology. 2004;34:159-167.
16. Haripriya R, Thirumalaivasan P. Isolation of cellulolytic bacteria and production of cellulase from coir pith, Int. J Res. Ins. 2017;4(1):13-23.
17. Irfan Muhammad, Asma Safdar, Quratulain Syed, Muhammad Nadeem. Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity, Turkish Journal of Biochemistry–Turk J Bio chem. 2012;37(3):287-293.
18. Kamara AH, Kamar S, Kamara MS. Effect of rice straw biochar on soil quality and the early growth and biomass yield of two rice varieties. Journal of Agricultural Sciences. 2015;6:798-806.
19. Kiio IK, Mulaa F, Jackim Wamalwa B, Munyali, Edward KM. Isolation and Characterization of a Thermos table Cellulase from Bacillus licheniformis Strain Vic Isolated from Geothermal Wells in the Kenyan Rift Valley. The Open Biotechnology Journal. 2016;10:198-207.
20. Kumar. Aerobic and Anaerobic Digestion of Agricultural Waste Followed by Vermicomposting and Enrichment; c2016.
21. Khaleel HL, Abd AN, Ali KM. Preparation of Nano-Cellulose from Industrial Waste by Ultrasonic Device. Journal of Biochemical Technology. 2018;9(1):35.
22. Krieg NR, Dobereiner J. Genus Azospirillum In: J. G. Holt and N. R. Krieg (Eds.) Bergey's Manual of Systematic Bacteriology Williams and Wilkins Baltimore, MD. 1984;1:94-104.
23. Li X, Gao P. Isolation and partial properties of cellulose-decomposing strain of *Cytophaga* sp. LX-7 from the soil. J Appl. Microbiol. 2008;82:73-80.
24. Loow YL, Wu TY, Md Jahim J, Mohammad AW, Teoh WH. Typical conversion of lignocellulosic biomass into reducing sugars using dilute acid hydrolysis and alkaline pretreatment. Cellulose. 2016;23:1491-1520. chttps://doi.org/10.1007/s10570-016-0936-8
25. Maki M, Leung KT, Qin W. The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. Int. J Biol. Sci. 2009;5:500-516.
26. Maki ML, Broere M, Tin Leung K, Wensheng Q. Characterization of some efficient cellulases producing bacteria isolated from paper mill sludges and organic fertilizers. International Journal of Biochemistry and Molecular Biology. 2011;2(2):146-154.
27. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 1959;31:426-428.
28. Ojumu T, Solomon V, Bamidele O, Betiku E, Layokun SK. Cellulase Production by Aspergillus flavus Linn Isolate NSPR 101 fermented in sawdust, bagasse and corncob. African J Biotechnol. 2003;2:150-152.
29. Piper CS. Soil chemical analysis Hans Publications, Bombay; c1966.
30. Pande VS. Recycling of organic wastes by fungi. M.Sc. (Agri.) Thesis (Unpub.). Dr. PDKV, Akola; c1978.
31. Ponnambalam AS, Deepthi RS, Ghosh AR. Qualitative Display and Measurement of Enzyme Activity of Isolated Cellulolytic Bacteria. Biotechnology and Bioengineering. 2011;1(1):33-37.
32. Pratima Gupta, Kalpana Samant, Avinash Sahu. Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential, International Journal of Microbiology. 2012, (5) Article ID 578925.
33. Shinde VS, Agrawal T, Kotasthane AS. Molecular Characterization of Cellulolytic Bacteria Derived From Termite Gut and Optimization of Cellulase Production, International Journal of Current Microbiology and Applied Sciences. 2017;6(10):2474-2492
34. Somani RB, Wangikar PD, Bhagwat VY, Raut BT. Study on decomposition of Agricultural waste by soil fungi. Food Farming and Agri. 1979;12(6):140-143.
35. Somani RB, Wangikar PD. Cellulolytic activity of some soil fungi. Food Farming and Agri. 1979;12(4):96-98.
36. Saini JK, Saini R, Tewari L. Lignocellulosic agriculture wastes as biomass feed stocks for second-generation bioethanol production: concepts and recent developments. Biotech. 2015;5:337-353. https:// doi.org/10.1007/s13205-014-0246-5 PMID: 28324547
37. Vimal J, Akhil V, Jini J. Isolation and Identification of Cellulose Degrading Bacteria and Optimization of the Cellulase Production, International Journal of Research in Biosciences. 2016;5(3):58-67.
38. Zaghoud L, Gouamid M, Benmenine A, Khanblouche A. Kinetic and Thermodynamic of Gentian Violet Removal by 2, 3-Dialdehyde Nano cellulose. Journal of Biochemical Technology. 2019;10(2):38-42.
39. Pointing SB, Buswell JA, Jones EG, Vrijmoed LL. Extracellular cellulolytic enzyme profiles of five lignicolous mangrove fungi. Mycological research. 1999 Jun 1;103(6):696-700.
40. Eriksson KE, Gupta JK, Nishida A, Rao M. Syringic acid metabolism by some white-rot, soft-rot and brown-rot fungi. Microbiology. 1984 Oct 1;130(10):2457-2464.