Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5888

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (5):** 1266-1272 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Screening of Lignocellulolytic Bacteria from the Soil Samples Collected From Different Geographical Locations of Chhattisgarh (C.G.), and Maharashtra (MH) India

R.M. Shinde[1*], V.S. Shinde[1], T. Agrawal[2] and A.S. Kotasthane[3]

[1]Ph. D. Scholar (Agri), [2]Senior Research Associate

Department of Plant Molecular Biology and Biotechnology, IGKV, Raipur-492012

[3]Professor and Head, Department of Plant Pathology, IGKV, Raipur-492012

*Corresponding Author E-mail: roshan.agricos@gmail.com

Received: 11.09.2017 | Revised: 18.10.2017 | Accepted: 23.10.2017

ABSTRACT

This study was aimed to screen the lignocellulolytic ability of Bacteria isolated from the soil samples collected from different geographical location of Chhattisgarh and Maharashtra State of India. The strains were screened for their ability to produce lignocellulolytic enzymes such as Cellulase, Lignin Peroxidase, And laccase using Carboxy methyl cellulose (CMC), Azure B, ABTS, and Guaiacol as a substrate. Further the isolates were characterised based on their utilization of sugar. The isolates then Screened for their ability to decompose Rice straw. This findings of the study showed that the Bacterial isolates with appreciable hydrolytic zones have good hydrolytic property of lignocellulosic agricultural waste.

Key words: *Bacteria, Lignocellulase, CMC ABTS, Guaiacol*

INTRODUCTION

The lignocellulose material of plant consists of three main compounds, namely cellulose, hemicellulose and lignin. After cellulose, lignin is the second most abundant renewable biopolymer in nature. It is most abundant aromatic polymer in the biosphere[9] thus; their usage is indispensable for the carbon cycle. Lignin being the most recalcitrant biomaterials on earth[6,12] prevents many microbes to get easy access to the cellulosic content of the plant cell wall, ultimately which delays rate of decomposition leading to difficulty in easy access to cellulose for microbes in nature. Every chemical compound is degraded by a range of microorganisms that turn out battery of enzymes that acts synergically. In the near future, processes that use lignocellulolytic enzymes are based on microorganisms could lead to new, environmentally friendly technologies. Such potent Bacteria can be very useful to manage tons of lignocellulosic biomass produces every year as a agriculture waste. Paddy straw is one of the most abundant lignocelluosic waste and huge amount of straw as solid biomass wastes produced annually[4].

Cite this article: Shinde, R.M., Shinde, V.S., Agrawal, T. and Kotasthane, A.S., Screening of Lignocellulolytic Bacteria from the Soil Samples Collected From Different Geographical Locations of Chhattisgarh (C.G.), and Maharashtra (MH) India, *Int. J. Pure App. Biosci.* **5(5):** 1266-1272 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5888

Rice Straw composition is predominantly cellulose with 32 to 47 %, hemicellulose ranging between 19 to 27% and lignin ranging between 5 to 24 %[10] .This leads to serious environmental pollution issues if untreated. There are some reports describing Bacteria releasing ligninolytic enzymes such as cellulase, Lignin peroxidase (LiP) and Laccase for degradation[5] .In this study, an investigation was attempted to isolate, and characterize potential lignocellulolytic bacterial strains.

MATERIALS AND METHODS

Collection of samples:

The soil sample used for the isolation of lignin degrading bacteria was collected from the diverse geographical region of Chhattisgarh and Maharashtra state India. Soil samples were collected during rainy season by digging soil 15 cm deep. Then soil sample was placed in sterilized self-sealing bags and kept at 4°C for further use.

Isolation of lignocellulose degrading bacteria:

Isolation was done by serial dilution method in which soil sample were serially diluted from 10^1 to 10^7 with distilled water and 0.1ml aliquots from each dilution was plated in mineral salt medium(MSM) of following composition (in g/l):Na2HPO4, 2.4; K2HPO4, 2.0; NH4NO3, 0.1; MgSO4,0.01; CaCl2, 0.01; D-glucose, 10.0; peptone, 3.0; Agar,15; at pH 7.0±0.2 and trace element solution (1 ml/l) amended with) 0.25% W/V Lignin. The samples were incubated at 30°C for a period of 7 days. The colonies that were phenotypically different was picked and purified by repeated streaking on the Nutrient agar plates. The purified strains were designated as AK1, AK2, AK15, AK17, AK21, S4, S7, S9, R13, R24, TR30, TR31, TR32, RDD, RDB, RDC, RDE, SH2, SH2A, SH4, GS2, GS4, GS5, GS7, GS11, GS16a, GS16b, U6, 16Aw (Table 1).

Screening of potential bacterial strains for cellulolytic and ligninolytic enzyme activity:

The isolated and purified bacterial strains were screened for cellulase, Lignin peroxidase and Laccase activity by plate assay method. Cellulolytic bacterial strains were identified by method described by Pointing (1999) using cellulose basal medium (C4HI2N206 5g; KH2PO4 1g; MgSO4.7H2O 0.5 g; Yeast extract 0.1 g; CaCl2.2H2O 0.001 g/liter) supplemented with 2 % w/v low viscosity CMC and 1.6% w/v agar and was autoclaved. Media prepared was transferred aseptically to sterile petridishes and allowed to cool. Then plates were inoculated with pure bacterial isolates and kept for incubation at 25°C in the darkness. Later on plates were stained with the 2 % w/v aqueous Congo red dye solution for 15 minutes, excess stain was pour off and destaining was done with 1M NaCI solution. Carboxymethylcellulose (CMC) is a substrate for Endoglucanase and so can be used as a test for Endoglucanase and glucosidase activity[1,2,7] . For the screening of ligninolytic bacterial strains LME Media with different substrate was used for screening. The substrate used for Lignin peroxidase was Azure B (0.002%). While Laccase activity was detected qualitatively in nutrient agar medium (NAM, NAM broth) containing (in g/l) peptone, 5.0; beef extract, 3.0; NaCl, 5.0; CuSO4 (1mM), (0.02%) ABTS (2, 2'azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) and guaiacol as a substrate (Chandra and Singh, 2012).

Biochemical characterization

Selected Bacterial isolates were then biochemically characterized using Hicarbohydrate™ kit to test carbon utilization profile as described by manufacturer (Himedia

Laboratories, Mumbai, India).Cell suspension of isolated Bacterial strains were prepared in Nutrient broth. An aliquot of 50 µl of already prepared suspension of screened isolates was inoculated to each well of Hicarbohydrate™ kit, and incubated at 28°C and kept for 24 hrs. The results were recorded according to the instructions given by the manufacturer.

Decomposition of rice straw using potential bacterial isolates

All the lignocellulolytic Bacterial isolates were tested for their ability to degrade rice straw. The experiment was set into disposable glass and petriplates. Rice straw was cut into 15 cm length and soaked in water so as to ensure even moisture followed by inoculation with bacterial cultures and it was kept for 30 days. Degradation ratio of rice straw was calculated according to a method described by Shamseldin and Abdelkhaldek[11] .The data was also taken for quantity of bacterial inoculums taken for each glass, weight of compost and percentage of degradation in each glass.

RESULTS AND DISCUSSION

Isolation and screening of Lignocellulolytic bacterial isolates:

The Lignocellulolytic Bacterial isolates were screened from soil samples collected from the different geographical locations of Chhattisgarh and Maharashtra. Out of 54 isolated bacterial strains 30 bacteria were selected by several screening methods by plate assay, to evaluate the lignocellulolytic properties like cellulolytic, and polyphenoloxidase activity. For cellulase activity CMCs plate assay was carried out. Positive strains were selected based on the observation of a yellow-opaque area around the bacterial growth against a red colour for undegraded CMC and diameter of hydrolysis zones were measured. Qualitative plate assay based on Azure B dye decolorization was carried out for screening lignin peroxidase activity and the positive strains were selected by examining halo zone around colonies. Further the isolates were screened for the production of laccase using Guaicol and ABTS as a substrate. Strains that had capacity to oxidize Guaicol present in media turned color of media to brown was considered positive for laccase. Similarly strains that oxidized ABTS into green oxidized zone around colonies was selected positive for Laccase production. (Fig 1)

Decomposition of rice straw using potential bacterial isolates

The potential of the isolated strains to decompose rice straw was assessed by testing their ability to utilize lignin so as to get easy access to cellulose for further deconstruction. All of the isolates were able to degrade rice straw by the 25th day (Fig 2). All the isolates showed degradation ratio between 85 to 93% (Fig 3) Out of selected 30 isolates SH2A isolate showed maximum degradation ratio about 93.97% followed by AK17 isolate 93.93%, AK 15 93.83%, U6 93.69% and SH2 93.66% isolate(Table 4th). W isolates showed lowest activity among all 30 isolates. In control, 59% of degradation ratio has been observed (Table 4th).Based on the observations, the bacterial isolates were found potential to degrade rice straw. These results are in same line with results obtained by[11] in their study of Isolation and identification of newly effective bacterial strains exhibiting great ability of lignin and rice straw biodegradation.

Table 1: List of bacterial isolates and their site collection used in the present study

Sr. No.	Bacterial isolate	Collection Site	GPS location
1.	AK1	Katepoorna forest Akola Maharashtra	N 20° 25 24.8 E 77° 11 40.6
2.	AK2	Katepoorna forest Akola Maharashtra	----------
3.	AK15	Katepoorna forest Akola Maharashtra	N 20° 25 18.1 E 77°11 4.5
4.	AK17	Katepoorna forest Akola Maharashtra	N 20° 25 25.7 E 77° 11 36.2
5.	AK21	Katepoorna forest Akola Maharashtra	N 20° 2515.5 E 77°11 42.3
6.	S4	Gangrel dam area C.G	N 20° 37 07.5 E 81°34 12.5
7.	S7	Gangrel dam area C.G	N 20° 37 11.1 E 81° 34 08.6
8.	S9	Gangrel dam area C.G	N 20° 37 08.4 E 81° 34 11.9
9.	SH4	Store house farmers hostel,IGKV.Raipur	N21° 14 02.0 E 81° 42 32.6
10.	SH2*	Store house farmers hostel,IGKV.Raipur	
11.	SH2A	Store house farmers hostel,IGKV.Raipur	
12.	R11	IGKV University field, Raipur	N 21° 14 09.8 E 81° 41 59.8
13.	R13	IGKV University field, Raipur	
14.	R24	IGKV University field, Raipur	
15.	RDD	Rice straw compost, IGKV , Raipur	N 21° 13 58 E 81° 41 50.0
16.	RDB	Rice straw compost, IGKV , Raipur	
17.	RDC	Rice straw compost, IGKV , Raipur	
18.	RDE	Rice straw compost, IGKV , Raipur	
19.	GS2A	Ghatarani forest C.G	N 20° 49 57.3 E 82° 03 27.9
20.	GS4A	Ghatarani forest C.G	N 20° 50 06.6 E 82° 03 27.9
21.	GS5A	Ghatarani forest C.G	N 20° 50 31.4 E 82° 03 39.8
22.	GS7	Ghatarani forest C.G	N 20° 50 36.8 E 82° 03 25.7
23.	GS11	Ghatarani forest C.G	N 20° 50 47.3 E 82° 03 18.3
24.	GS 16 A	Ghatarani forest C.G	_____
25.	GS16B		
26.	U6	IGKV,Field	N 21° 14 05 E 81° 42 02.3
27.	16 A W	Rice straw compost	N 21° 14 06 E 81° 42 52.1
28.	TR 30	Gariyaband (Joba)forest	N 20° 31 018 E 082° 07 473
29.	TR 31	Gariyaband (Joba)forest	N 28° 31 018 E 082° 07 488
30.	TR 32	Gariyaband (Joba)forest	N 28° 35 402 E 080° 02 131

Table 2: Estimation of CMCase, lignin peroxidase, and Laccase positive strains using CMC, Azure B ABTS, and Guaiacol as a substrate

Sr. no	Bacterial strain	Lignocellulolytic enzymes				
		CMCase	CMCase assay (zone diameter in mm)	Lignin peroxidase	Laccase (ABTS)	Laccase (Guaicol)
1	AK 1	+	7	+	+	+
2	AK2	+	5	+	-	-
3	AK15	+	10	+	+	+
4	AK17	+	12	+	+	+
5	AK21	+	10	+	+	+
6	S4	+	8	-	+	+
7	S7	+	8	-	+	+
8	S9	+	6	+	+	+
9	R11	+	9	+	+	+
10	R13	+	7	-	+	+
11	R24	+	10	+	+	+
12	TR 30	+	10	-	+	+
13	TR31	+	10	+	+	+
14	TR32	+	10	+	+	+
15	RDD	+	9	-	+	+
16	RDB	+	8	-	+	+
17	RDC	+	9	+	+	+
18	RDE	+	7	+	+	+
19	SH4	+	9	-	+	+
20	SH2*	+	6	-	+	+
21	SH2A	+	9	+	+	+
22	GS2	+	8	-	+	+
23	GS4	+	7	-	+	+
24	GS5	+	10	+	+	+
25	GS7	+	10	-	+	+
26	GS11	+	6	-	+	+
27	GS16a	+	8	-	-	+
28	GS16b	+	8	-	-	+
29	U6	+	8	-	+	+
30	16Aw	+	6	+	-	+

Table 3: Distinct characteristics Preference for utilization of carbon source revealed by 30 lignocellulolytic selected bacterial isolates

Test	AK1	AK15	AK17	AK21	S4	S7	S9	R11	R13	R24	SH4	SH2*	SH2A	TR30	TR32	RDD	RDB	RDC	RDE	GS2	GS4	GS5	GS7	GS11	GS16A	GS16B	U6	W
1	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-
2		+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
3	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	-
4	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
6	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-
7	+	+	+	-	+	-	+	+	+	+	+	+	-	+	+	+	-	+	-	-	+	+	+	+	+	+	+	-
8	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	+	+	+	+	-
9	+	+	+	-	+	-	+	-	+	+	+	+	-	+	+	-	-	+	+	-	+	+	-	+	+	+	+	+
10	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	-	+	+	+	+	+	+	+	-
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	-
12	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-
13	+	+	-	-	+	-	+	+	-	+	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-
14	+	-	-	-	+	-	+	+	-	+	-	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-	-
15	+	-	-	-	-	+	-	-	+	+	-	+	+	+	-	+	+	+	-	-	-	-	-	+	-	-	+	+
16	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	+	-
17	+	-	-	-	+	-	-	+	-	-	-	-	+	-	-	+	+	-	+	-	-	+	_	+	+	+	-	-
18	+	-	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-	+
19	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+
20	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
21	+	-	-	-	+	-	-	-	-	+	-	-	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+
22	+	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	+	-
23	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	+	-	-	+	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	-
25	+	-	-	-	-	-	-	+	-	+	-	+	+	+	-	+	-	+	+	-	+	-	_	+	+	-	+	-
26	+	-	-	+	+	-	+	+	+	-	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	-
27	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
30	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
31	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32	+	+	-	+	+	-	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	-
33	-	-	-	-	-	-	+	-	+	+	-	+	+	-	+	+	+	-	+	-	-	-	-	-	-	-	+	+
34	-	-	-	-	-	-	+	-	+	-	+	+	+	+	-	+	+	-	-	-	+	-	-	-	-	-	+	+
35	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-

Table 4: Degradation ratio of rice straw after inoculating with potential isolates

Bacterial isolate	Initial weight of rice straw taken	Distilled water added	Amount of bacterial culture added	Weight after adding bacterial culture	Dry weight after 25 days	Degradation (%)
Control	10g	10 ml	-	-	8.2	59
AK1	10g	10 ml	10ml	35±5	4.86	86.11
AK 15	10g	10 ml	10ml	35±5	2.16	93.83
AK 17	10g	10 ml	10ml	35±5	2.12	93.94
AK21	10g	10 ml	10ml	35±5	2.88	91.77
S4	10g	10 ml	10ml	35±5	3.2	90.86
S7	10g	10 ml	10ml	35±5	3.84	89.03
S9	10g	10 ml	10ml	35±5	4.2	88.00
R11	10g	10 ml	10ml	35±5	3.44	90.17
R13	10g	10 ml	10ml	35±5	2.56	92.69
R24	10g	10 ml	10ml	35±5	3.22	90.80
SH4	10g	10 ml	10ml	35±5	3.15	91.00
SH2	10g	10 ml	10ml	35±5	2.22	93.66
SH2A	10g	10 ml	10ml	35±5	2.11	93.97
TR 30	10g	10 ml	10ml	35±5	2.65	92.43
TR32	10g	10 ml	10ml	35±5	2.59	92.60
RDD	10g	10 ml	10ml	35±5	4.55	87.00
RDB	10g	10 ml	10ml	35±5	4.26	87.83
RDC	10g	10 ml	10ml	35±5	2.24	93.60
RDE	10g	10 ml	10ml	35±5	4.22	87.94
GS2B	10g	10 ml	10ml	35±5	3.37	90.37
GS4A	10g	10 ml	10ml	35±5	3.99	88.60
GS5A	10g	10 ml	10ml	35±5	3.16	90.97
GS7	10g	10 ml	10ml	35±5	3.14	91.03
GS11	10g	10 ml	10ml	35±5	3.44	90.17
GS16A	10g	10 ml	10ml	35±5	5.1	85.43
GS16B	10g	10 ml	10ml	35±5	5.35	84.71
U6	10g	10 ml	10ml	35±5	2.21	93.69
W	10g	10 ml	10ml	35±5	5.92	83.09

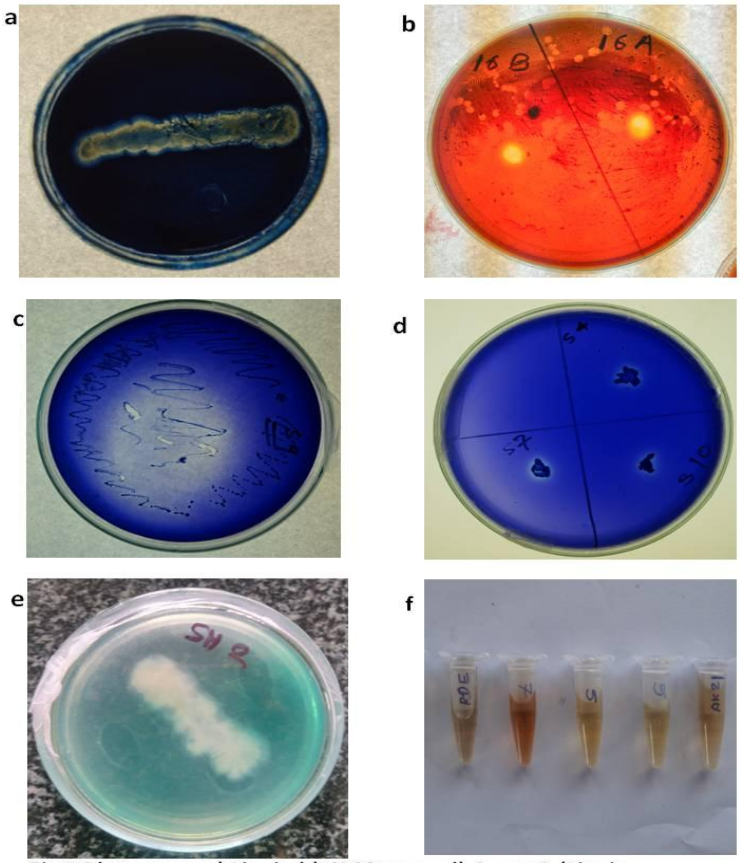

Fig.1 Plate assay a) Lignin b) CMCase c , d) Azure B (Lignin peroxidase) e) ABTS(Laccase) f) Guaiacol (Laccase)

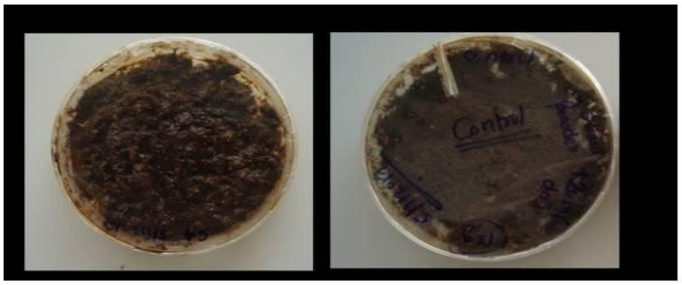

Fig 2 : Rice straw decomposition using isolated bacteria

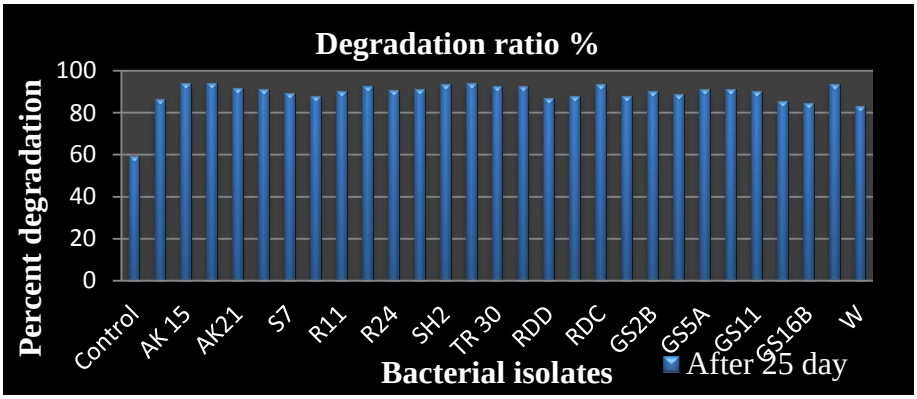

Fig 3: Degradation ratio of Rice straw shown by potential isolates

CONCLUSION

In Present study the lignocellulolytic bacteria was isolated from the soil samples collected from different geographical regions of Chhattisgarh and Maharashtra region. The isolated strains were further screen for their ability to produce cellulase, lignin peroxidase and laccase activity using CMC, Azure B, Guaiacol and ABTS as a substrate. The laccase activity of 30 bacterial isolates was screened in the Nutrient broth supplemented with 2mM Guaicol. Out of 30 bacterial isolates 28 isolates was found positive for the production of cellulase, lignin peroxidase, and laccase based on the halo zone formation and oxidation pattern of their respective substrate viz. CMC, Azure B Guaiacol and ABTS. Selected Strains were used to inoculate rice straw for decomposition. All of the isolates were able to degrade rice straw by the 25^{th} day in lab condition. All the isolates showed degradation ratio between 85 to 93% (Table 4) Out of selected 30 isolates SH2A isolate showed maximum degradation ratio about 93.97% followed by AK17 isolate 93.93%, AK 15 93.83%, U6 93.69% and SH2 93.66% isolate. All the selected 30 Bacterial isolates has their preference over the utilization of different carbon source among all GS11, U6, RDC, SH4, AK1 can only efficient to use Lactose as a carbon source. No isolate found to be positive for erythritol utilization.Xylitol was used by only SH4, U6 and W isolate. All the isolates were found positive for the esculin hydrolysis except AK 17, S4 and S7.No isolates were found having ability to utilize α methyl D manoside. (Table 3)

REFERENCES

1. Buswell, lA., Cai, YJ., Chang, S.T., Peberdy, IF., Fu, S.Y. and Yu, H.S. Lignocellulolytic enzyme profiles of edible mushroom fungi. World *Journal of Microbiology and Biotechnology* **12:** 537-542 (1996).
2. Cai, YJ., Buswell, lA. , Chang and S.T. . Cellulases and hemicellulases of Volvariella volvacea and the effect of tween 80 on enzyme production. *Mycological Research* **98:** 1019-1024 (1994).
3. Chandra, R., and Singh R. Decolorization and detoxification of black liquor from rayon grade pulp paper mill effluent by mixed bacterial culture isolated from pulp paper mill effluent polluted site. *Biochemical Engineering Journal*, **61:** 49-58 (2012).
4. Chang, K.L., Thitikorn-amorn, J., Hsieh, J.F., Ou, B.M., Chen, S.H., Ratanakhanokchai, K., Huang, P.J.,Chen and S.T. . Enhanced enzymatic conversion with freeze pretreatment of rice straw. *Biomass Bioenergy*. **35:** 90–95 (2011).
5. Naz, S. Isolation and Characterization of Ligninolytic Bacteria from Bharda Khar Agro Field of Bhilai-Durg.Online International *Interdisciplinary Research Journal*, **4:**156-166 (2014).
6. Perez, J., Rubia, T.D.L., Martinez, J. and Kapley A. Biodegradation and biological treatment of cellulose, hemicellulose and lignin: An overview. *Int. Microbiol.* **5:** 53-63 (2001).
7. Pointing, S.B., Buswell, lA., Vrijmoed, L.L.P. and Jones, E.B.G.. Extracellular cellulolytic enzyme profiles of five lignicolous mangrove fungi. *Mycological Research* **103:** (In press) (1999a).
8. Pointing and S.B. An assessment of ligninolytic potential in basidiomycetes from Hong Kong forests. *Microbial Ecology* **25:** (In press) (1999).
9. Rahman, N.H.A., Rahman, N.A.A., Surainiabdaziz M. and Hassan M. Production of ligninolytic enzymes by newly isolated bacteria from palm oil plantation soils. *Bioresour*. **8(4):** 6136-6150 (2013).
10. Roberto IC, Mussatto SI, Rodrigues RCLB, "Dilute-acid hydrolysis for optimization of Ronald Hatfield and Romualdo S. Fukushima Can Lignin Be Accurately Measured? *Cropsci*. **45(3):** 832-839 Received: (2004).
11. Shamseldin, A and Abdelkhalek , A.A. . Isolation and identification of newly effective bacterial strains exhibiting great ability of lignin and rice straw biodegradation. *Int .J. Curr. Microbiol*. App. Sci**. 4(6):** 1039-1049 (2015).
12. Zhang, Y.H.P., Lynd and L.R. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplex cellulose systems. *Biotechnology* and *Bioengineering*. **88:**797-824 (2004).