SCREENING OF CELLULOSE PRODUCING BACTERIA FROM SOIL AND OPTIMIZATION OF MEDIUM FOR ENHANCING CELLULASE ACTIVITY.

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Abstract:
Biomedical waste demarcates one of the most common waste components which is emerging on an alarming rate especially due to a rapid increased Healthcare sector and an increase in number of healthcare facilities in India. It has been a greater concern to find out some generous methodologies to systematically monitor the accumulated wastes in facilities and also it has become quite important to develop processes for decomposing and degrading these Biomedical wastes. Within this contrast an important concern is degrading a huge number of cellulosic wastes which could be in form of Cotton swabs, Dressing Bandages, Tissue Papers and other wastes. As the main component of these basic Biomedical wastes is Cellulose, so the prime focus of the work hereby is to screen the Microbes which are capable of Producing Bacteria and also Optimizing the medium for enhancing the cellulose activity. Among the 5 isolates taken it was found that only 2 isolates i.e., CL 002 and CL005 were showing significant selectiveness for Cellulase Producing Bacteria as per the results obtained.

Key words: Cellulase, Isolates, Screening, optimization, Enzyme

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Introduction:

Cellulose is a Naturally existing organic compound mount in nature in a huge which consists of thousands of carbon, hydrogen and oxygen atoms. It comprises of Almost all the cell wall of Plant Cells Existing on earth. Due to this Cellulose earned a very dominating position as Biomolecule Governing structural component of a huge amount of Biomass on earth. Beyond this Celluolse serve as dietary suppiment in various Forms for both humans and animals in various forms. Celluose do serve as an important component in day-to-day life which may be Textile, Clothing, Healthcare products etc. But with an increased Celluosic content in Regular Biomedical waste. It has become quite crucial to work on factors and methods to Manage this waste by a proper and safety processes. This could involve isolating cerating bacterias having cellulose Degrading properties via Cellulase enzymes produced by them.

From past a lot of Researches have shown that, cellulase enzyme has long been involved in synthesizing fermentable sugars from lignocellulose biomass. Cellulases are usually made up

of three groups of enzymes that work together: endoglucanases hydrolyzed the polymeric chain of cellulose, exoglucanases that aid in the release of cellobiose from the cellulose polymer, and $\beta\beta$-glucosidases, which assist in the conversion of cellobiose to glucose.
Global Cellulase's Market Report for 2018 reveals that Asia Pacific is the largest purchaser of cellulases and added that cellulase application would hit USD 2300 million by 2025, which will increase by 5.5% over the 2018-2025 period, at an annual CAGR (compound annual growth rate) (1).

The synthesis of cellulase enzymes in the coming time seems to be most exciting advanced field of research by biotechnology with several factors, including cost, genetic engineering, a particular activity, and purification steps and advance treatment steps.
The degradation of cellulosic wastes from a general Healthcare facility has been identified in numerous investigations. Still there exist a Huge Challenge for the scientific community to work on an effective Bacterial Isolates which could be fruitful enough to degrade Cellulosic waste to a large extent. For this reason, there is a growing demand for the isolation of bacterial aerobic strains, which reasonably produce higher cellulase activity. Our study has conducted by isolating aerobic bacteria from a local soil sample that could Isolate Bacteria and study its Morphological and Biochemical study to further screen it so that it can be utilized for Cellulase production

Materials and Methods:
Isolation of Bacteria:
The Basic source for sample collection was the dump areas from Backyard of KSV College Bijnor. Collected sample (1 g) was led to serial dilution.
Traditional serial dilution technique has been used to isolate cellulolytic bacteria through agar plate about 10^{-1} to 10^{-10} serial Dilution. [2]. 5 Inoculates after Serial Dilution were used for inoculation and 100 µl of the solution from concentration transferred into Petri dishes containing Carboxy methyl cellulose (CMC) agar media plates containing 0.5 g KH2PO4, 0.25 g MgSO4, 0.25 g cellulose and 2 g gelatin for the enhancement of the bacterial activity. [2]. The CMC agar plates were subsequently incubated for 24 hours at 37°C. Different discrete colonies were found on an agar plate and were selected further.

Primary Screening of Cellulose Degrading Bacteria:
CMC agar plates had been soaked within congo red solution for about 5 minutes and then allowed to stand at room temperature. Out of the 5 Plates 2 Have shown a clear Zone pn 1.0 % carboxymethylcellulose, 0.1% KH2PO4, 0.005% NaCl, 0.04 % MgSO4.7H2O, 0.1 % K2HPO4, 0.000125% FeSO4, 1.8 % agar, pH 7.0 and incubate at 37°C for 72 hours.

Identification of Isolates:
The identification of the desired isolates was done by doing morphological and Biochemical examination, which emphasized on, Pure Culture of Positive Isolates, Morphology, Biochemical Test: Catalase Test, Citrate utilization test, Gelatin Hydrolysis Test, Starch Hydrolysis Test, Carbohydrate Utilization, Methyl Red and Voges Proskauer Test and thus determining the specificity of the 2 isolates on basis o there testing.

Synthesis of Enzyme:

In a conical flask with a capacity of 100 mL, ten mL of medium were withdrawn. After being sterilized in an autoclave at a temperature of 121°C for fifteen minutes, the flasks were allowed to cool before being inoculated with a bacterial culture that had grown overnight. After being inoculated, the medium was shaken for 24 hours at 37°C inside an incubator. After the fermentation process was complete, the culture medium was centrifuged at 5000 rpm for 15 min in order to obtain the crude extract, which was used as an enzyme source.

Production medium (g/L):

Sl. No.	Composition	Amount (g/L)
1	Glucose	0.5 gm
2	Peptone	0.75 gm
3	$FeSO_4$	0.01 gm
4	KH_2PO_4	0.5 gm
5	$MgSO_4$	0.5 gm

Cellulase Enzyme Assay:

The DNSA method was used to determine how much reducing sugar was liberated during the hydrolysis. Under the following standard assay conditions, the enzyme unit (EU) was calculated as the amount of cellulase required to release 1 mole of reducing sugar per ml per minute. [3]

Optimization Process for Maximum Cellulase Production:

Cellulase Production was done and observed within different Physiological Conditions and thus the medium optimization was done. It plays a significant role in producing developing a media which can give on a sufficient amount of desired Enzyme. Ph, Temperature, Carbon source, Nitrogen source, Agro-based waste material served as an important factor to boost up the efficiency of media and get a Desired constituent acting as a satisfactory growth Medium.

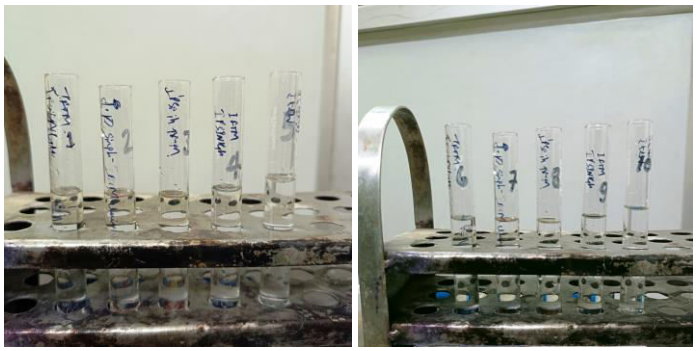
Result and Discussion:

Isolation and Primary Screening for Cellulase Producing Bacteria:

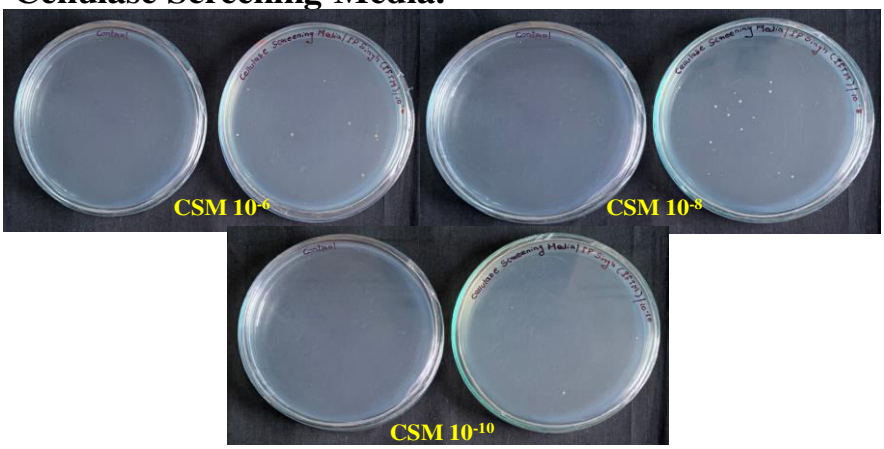
In this analysis, 2 samples were collected from KSV College Bijnor, yielding 10 isolates from each sample using the dilution 10^{-1} to 10^{-10}. Out of these 5 Were observed showing a Comparative growth on Agar plates and were reconfirmed for Cellulosic activity by zones observed after using Congo Red.

Colony Morphological and Biochemical Characterization:

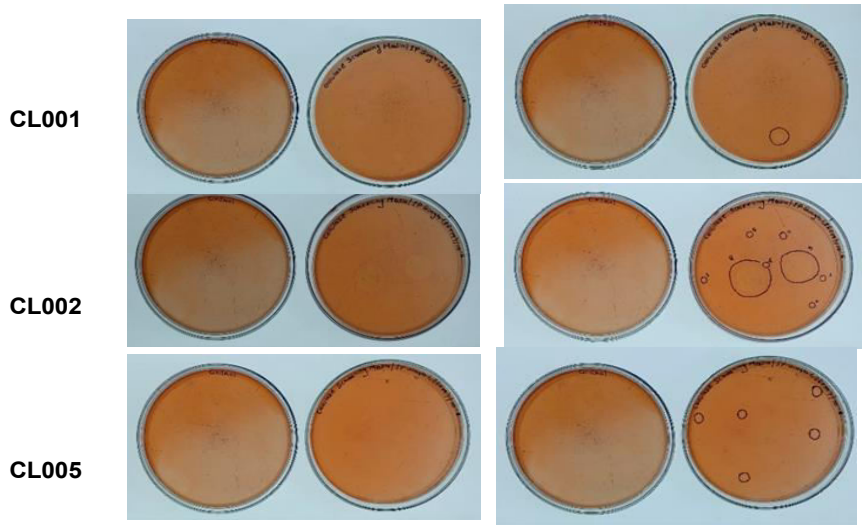
Serial Dilution



Petri Plates with Growth Observed after Inoculating with 100 ul Serial Diluted Sample IN Cellulase Screening Media.



Isolation and Primary Screening of Cellulase Enzyme Producing Microbes



Morphology and Biochemical results of Isolate 1: CL002:

Table 1: Colony appearance of isolate 1 (CL002):

Sl. No.	Shape	Size	Structure	Texture	Appearance	Color	Code
1	Round	Small	Round	Smooth	Creamy	White	CL002

Table 2: Observation and Inference of Biochemical tests of isolate 1 (CL002):

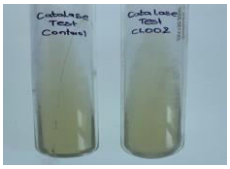
Sl. No.	Test	Observation	Test Result	Inference
1	**Catalase test**	No Bubble formation	-ve	Not a Catalase producing Bacteria
2	**Citrate utilization test**	Color change	+ve	Capable of Fermenting Citrate
3	**Gelatin Hydrolysis Test**	Liquefaction of media	-ve	Not a Gelatinases producing bacteria
4	**Starch Hydrolysis Test**	halo zone	+ve	Amylase producing
5	**Indole Test**	Brown ring	-ve	Unable to decompose tryptophane to indole
6	**Gram's staining**	Purple Colour/round shape	+ve	Gram +ve; coccus
7	**Carbohydrate Utilization Test**	Glucose- Pink to orange colour change	(A/NG)* Weak +ve	Glucose fermenting
		Lactose- Pink to orange	(A/NG)* Weak	Lactose fermenting

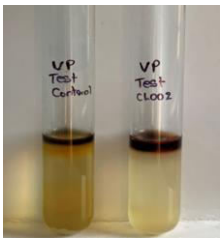
			+ve	
		Sucrose- Pink to yellow	(A/NG)* Strong +ve	Sucrose fermenting
8	**Methyl Red**	Red colour ring appearance	+ve	Glucose fermenting with mixed acid formation
9	**Voges Proskauer Test**	No cherry red ring appearance	-ve	No acetyl methyl carbinol

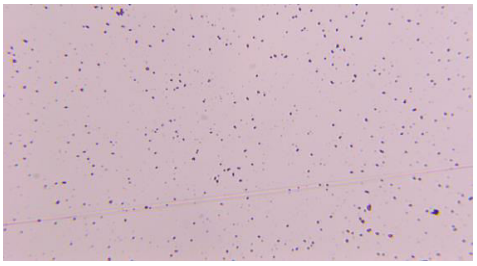
**A: Acid producing; NG: Non-gas producing*

Results CL002

PURE CULTURE







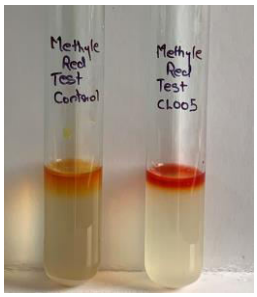
Gram Staining

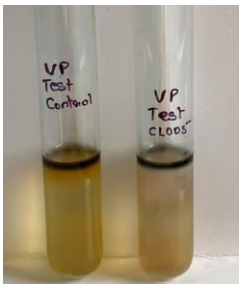
Morphological and Biochemical Testing of Isolates CL005

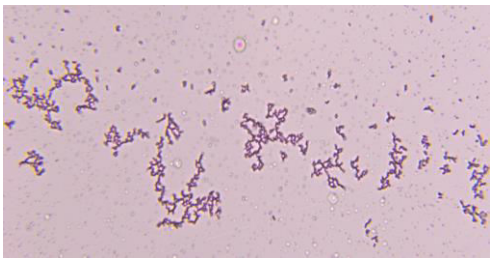
S.No	Shape	Size	Structure	Texture	Appearance	Color	Code
2	Round	Small	Round	Smooth	Creamy	White	CL005

S. No	Test	Observation	Test Result	Inference
1	Catalase test	Bubble formation	+ve	Catalase producing
2	Citrate utilization test	No Colour change	-ve	Non-Fermentative
3	Gelatin Hydrolysis Test	No Liquefaction of media	-ve	Not a Gelatinases producing bacteria
4	Starch Hydrolysis Test	Halo zone	+ve	can hydrolyze starch
5	Indole Test	Cherry red ring	+ve	Has ability to decompose tryptophane to indole
6	Gram's staining	Purple colour/ round shape	+ve	Gram positive; Streptococcus
7	Carbohydrate Utilization Test	Glucose- Pink to yellow	(A/NG)* Strong +ve	Weak Glucose fermenting
		Lactose- Pink to yellow	(A/G)* Strong +ve	Weak Lactose fermenting
		Sucrose- No colour change	(NA/NG)* -ve	Non sucrose fermenting
8	Methyl Red	Red colour ring appearance	+ve	Glucose fermenting with mixed acid formation
9	Voges Proskauer Test	No cherry red ring appearance	-ve	No acetyl methyl carbinol

RESULTS CL005







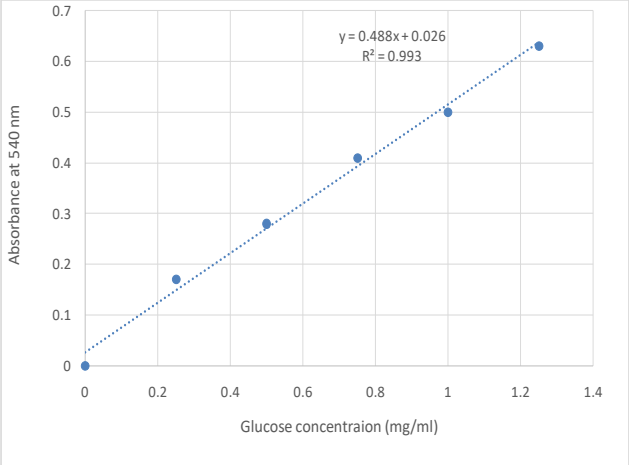
GRAM STAINING

ENZYME PRODUCTION FLASK VESSEL

Extraction of Enzyme:

ENZYME ASSAY

S.No.	Concentration (mg/ml)	Absorbance at 540 nm
1	0	0
2	0.25	0.17
3	0.5	0.28
4	0.75	0.41
5	1	0.5
6	1.25	0.63



Optimization of Media:

The media optimization was done Considering various Physiological parameters to enhance the enzyme content and its activity.

Table: Result recorded for Optimization of pH- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005:

Sl. No.	Parameter	Sample	pH	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	pH	CL002	7	0.11	0.171	0.126
2		CL002	8	0.38	0.724	0.536
3		CL002	9	1.21	2.425	1.795
4		CL002	10	1.28	2.568	1.901
5		CL002	11	0.27	0.499	0.369
1		CL005	7	0.09	0.130	0.096
2		CL005	8	0.17	0.294	0.217
3		CL005	9	1.35	2.712	2.007
4		CL005	10	1.33	2.671	1.977
5		CL005	11	0.93	1.851	1.370

Table: Result recorded for Optimization of Temperature- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005:

Sl. No.	Parameter	Sample	Temp (°C)	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	Temperature	CL002	35	1.11	2.220	1.643
2		CL002	40	1.28	2.568	1.901
3		CL002	45	0.75	1.482	1.097
4		CL002	50	0.54	1.052	0.778
5		CL002	55	0.47	0.908	0.672
6		CL002	60	0.39	0.744	0.551
1		CL005	35	1.31	2.630	1.946
2		CL005	40	1.45	2.917	2.159
3		CL005	45	0.88	1.749	1.294
4		CL005	50	0.65	1.277	0.945
5		CL005	55	0.41	0.785	0.581
6		CL005	60	0.37	0.703	0.521

Table: Result recorded for Optimization of Carbon source- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005:

Sl. No.	Parameter	Sample	Carbon source	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity

						(U/mL)
1	Carbon source	CL002	Starch	1.15	2.302	1.704
2		CL002	Glucose	1.93	3.900	2.887
3		CL002	Maltose	1.04	2.076	1.537
4		CL002	Lactose	0.91	1.810	1.340
5		CL002	Fructose	0.83	1.646	1.218
1		CL005	Starch	1.85	3.736	2.765
2		CL005	Glucose	2.01	4.064	3.008
3		CL005	Maltose	1.54	3.101	2.295
4		CL005	Lactose	1.6	3.224	2.386
5		CL005	Fructose	1.22	2.445	1.810

Table: Result recorded for Optimization of Nitrogen source- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005:

Sl. No.	**Parameter**	**Sample**	**Nitrogen source**	**Absorbance at 540 nm**	**Glucose released (mg/ml)**	**Enzyme activity (U/mL)**
1	Nitrogen source	CL002	Yeast extract	1.86	3.757	2.780
2		CL002	Peptone	1.73	3.490	2.583
3		CL002	Urea	1.66	3.347	2.477
4		CL002	Ammonium Sulphate	1.41	2.835	2.098
1		CL005	Yeast extract	1.62	3.265	2.416
2		CL005	Peptone	1.84	3.716	2.750
3		CL005	Urea	1.33	2.671	1.977
4		CL005	Ammonium Sulphate	1.31	2.630	1.946

Table: Result recorded for Optimization of Agri-waste substrate- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005:

Sl. No.	**Parameter**	**Sample**	**Agri-waste substrate**	**Absorbance at 540 nm**	**Glucose released (mg/ml)**	**Enzyme activity (U/mL)**
1	Agri-waste substrate	CL002	Groundnut cake	0.64	1.257	0.930
2		CL002	Coconut cake	0.52	1.011	0.748
3		CL002	Soy cake	0.41	0.785	0.581
4		CL002	Wheat bran	0.78	1.544	1.142
1		CL005	Groundnut cake	0.34	0.642	0.475
2		CL005	Coconut cake	0.21	0.376	0.278
3		CL005	Soy cake	0.27	0.499	0.369

4		CL005	Wheat bran	0.73	1.441	1.067

Table: Result recorded for Optimization of Different concentration of Optimized C-source- Glucose released and enzyme activity (U/mL) for isolates CL002 and Cl005:

Sl. No.	Parameter	Sample	% C-source (Glucose)	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	Concentration of C-Source (Glucose)	CL002	1	0.15	0.253	0.187
2		CL002	2	0.18	0.314	0.232
3		CL002	3	1.63	3.285	2.432
4		CL002	4	1.72	3.470	2.568
5		CL002	5	1.82	3.675	2.720
1		CL005	1	0.09	0.130	0.096
2		CL005	2	0.07	0.089	0.066
3		CL005	3	1.15	2.302	1.704
4		CL005	4	1.47	2.958	2.189
5		CL005	5	1.59	3.203	2.371

Enzyme activity calculation at Optimized condition:

Table: Enzyme activity (U/mL) for isolates CL002 and Cl005 under Optimized conditions

Sl. No.	Sample code	Conditions	Absorbance at 540 nm	Glucose released (mg/ml)	Enzyme activity (U/mL)
1	CL002	pH-10; 40°C; N-source- yeast extract	2.24	4.699	3.478
2	CL005	pH-9; Temp-40°C; N-source- peptone	2.15	4.351	3.220

Conclusions:

A wide range of cellulolytic bacteria can be found in soil, Dump areas and waste materials surfaces too including those that have yet to be isolated. This research was mainly focused to isolate potential cellulase-producing bacterial isolates from Dump area soil samples. Selectively isolates were isolated based on their Biochemical activities and Physiological Characteristics and cellulolytic properties. Out of the isolates taken on CL002 and CL005 were having cellulose producing properties.

The Cellulase production media were rather optimized to obtain a Quality and Quantity based Enzyme which could be beneficial with a point of Cellulase Degrading properties.

The Enzyme activity was observed in Optimized specific Conditions of pH-10; 40°C; N-source- yeast extract and was found 3.478 U/mL for CL002 and 3.220U/mL for CL005.

Further Large-scale studies are to be made based on Molecular approaches so as to enhance the Quality and Quantity of Cellulase Enzyme which could be beneficial in various sectors like Textile Healthcare and other Industries.

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