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Isolation and Purification of Cellulase Enzyme from *Triticum Aestivum*

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Abstract – The aim of the present study was to isolate and purify cellulase enzyme from Triticum aestivum cultivar rhizosphere and canal soil. The sample was collected under septic condition in sterile bag. A total of 15 isolates were identified for cellulase production in which 10 isolates from the canal soil and 5 isolates from the canal root soil. These microbial isolates were checked for their identity and similarity among themselves and similarly looking microbes were discarded and only one representing strain from each group was preserved for future use.

Keyword: Cellulase, Isolation, Industrial, Application

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INTRODUCTION

Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoan's that catalyze cellulolysis (i.e. the hydrolysis of cellulose). The EC number for this group of enzymes is EC 3.2.1.4. It is derived from D-glucose units, which condense through β(1→4)-glycosidic bonds (Yakubu et. al., 2011). Cellulose has no taste, is odourless & is hydrophilic (Ghosal et. al., 2011). The biological degradation of cellulose has been studied for many years, and a number of cellulolytic enzymes, especially cellulases produced by fungi and bacteria, have been isolated and characterized (Tomme, et. al., 1995). Five general types of cellulases based on the type of reaction catalyzed:

1.1. Endocellulase breaks internal bonds to disrupt the crystalline structure of cellulose and expose individual cellulose polysaccharide chains

1.2. Exocellulase cleaves two to four units from the ends of the exposed chains produced by endocellulase, resulting in the tetrasaccharides or disaccharides, such as cellobiose. There are two main types of exocellulases [or cellobiohydrolases (CBH)] - CBHI works processively from the reducing end, and CBHII works processively from the non-reducing end of cellulose.

1.3. Cellobiase or beta-glucosidase hydrolyses the exocellulase product into individual monosaccharides.

1.4. Oxidative cellulases depolymerize cellulose by radical reactions, as for instance cellobiose dehydrogenase (acceptor).

1.5. Cellulose phosphorylases depolymerize cellulose using phosphates instead of water.

Cellulase has application in various industries for example Pulp and Paper Industry, Textile Industry, Bio-ethanol Industry, Wine and Brewery Industry, Food Processing Industry, Animal Feed Industry, Agricultural Industries, Olive Oil Extraction, Carotenoid Extraction, Detergent Industry, Waste Management. From the last decade the application of cellulase in pulp and paper industry is increase (Kues and Militz, 2004). It is also reported in bleachability of softwood kraft pulp give more brightness score as compared to xylanase treatment (A. Singh, et. al., 2007, Suominen and Reinikainen, 1993). Cellulase also use in enzymatic deinking (Kibblewhite, et. al., 1995). These enzyme also use in sanitary paper (Hsu and Lakhani, 2002), production of biodegradable cardboard (Buchert, et. al., 1998) and paper towels (Salonen, 1990).

The main use of cellulase enzyme in textiles is to improved hand and appearance in wet textile processing for finishing of cellulose-based textiles. cellulase also use in removal of dirt particles trapped within the microfibril network and softening the garments (Hebeish and Ibrahim, 2007). In the recent year the cellulase most used in the biofuel production by the saccharifiaction of lignocellulosic materials of as sugarcane bagasse, corncob, rice straw, Prosopis juliflora, Lantana camara, switch grass, saw dust, and

forest (Gupta, et. al., 2011). Cellualse also use in production of wine and beers by the fermentation processes and improve both quality and yields of the fermented products (Bamforth, 2009). Cellulase have a wide range of use in food and biotechnology industry. It is used for extraction and clarification of fruit and vegetable juices to increase the yield of juices by macerating enzyme complex (cellulases, xylanases, and pectinases) (Carvalho, et. al., 2008). Cellulases and hemicellulases have received considerable attention in the feed industry due to improve feed value and performance of animals. By the use of cellulases or xylanases improve the nutitional value of feed (Dhiman, et. al., 2002). By the different combinations of cellulases, hemicellulases, and pectinases in agriculture use for enhancing the growth of crops and controlling plant diseases. Cellulases and related enzymes from certain fungi protect the plant from disease by degrading the cell wall of plant pathogens. Cellulases have it is also used for the improvement of the soil quality and reduce dependence on mineral fertilizers (Ortiz Escobar and Hue, 2008, Tejada, et. al., 2008). Due to the numerous health claims of olive oil, the extraction of olive oil has attracted the interest of international market. In the Extraction of olive oil involves three step (1) crushing and grinding of olives in a stone or hammer mill (2) passing the minced olive paste through a series of malaxeurs and horizontal decanters (3) high speed centrifugation to recover the oil. The high quality of olive oil is produced in cold pressing condition by the use of freshly picked, clean, and slightly immature fruits (Galante, et. al., 1998).

Cellulase are used in carotenoid extraction. Carotenoids are responsible for many plant colors from red to yellow. There is a continuously growing market for carotenoids as food colorants due to their desirable properties, such as their natural origin, null toxicity, and high versatility, providing both lipo- and hydrosoluble colorants with colors ranging from yellow to red. Provitamin A activity, a role in lipid oxidation, and anti-carcinogenic properties have very important biological functions of carotenoid pigment (Inar, 2005). In the detergents industry is a more recent innovation the use of cellulases along with protease and lipase. Cellulase preparations improve color brightness, feel, and dirt removal from the cotton blend garments. Alkaline cellulases as a potential detergent contact the cellulose within the interior of fibers and remove soil from the inter-fibril spaces (5). The wastes generated from agricultural fields, agro-industries and forest causing environmental pollution because of contain a large amount of unutilized or underutilized cellulose (Milala, et. al., 2005). In the recent year these wastes are utilized to produce valuable products such as animal feeds, human nutrients, sugar, biofuels, enzymes, cheap energy sources for fermentation (Gupta, et. al., 2010, Gupta, et. al., 2010, Kuhad, et. al., 2010, Humpf and Schreier, 1991)

MATERIAL AND METHODS

Yeast extract (Bacteriological grade), Cellulose, Tryptone ((Bacteriological grade), Sodium Chloride (GR), Iodine, Distilled Water

Sample Collection

Wheat rhizosphere samples were collected under a septic condition in sterile bag (Himedia Pvt ltd, India). Physiological parameter like pH, Temperature, texture, color was observed of each sample. Samples were transported to lab and stored at 4**°C** till processing.

Figure 3.1: **Sample collection**

Medium Preparation

The microbial growth medium was prepared as per the given composition and the steps mentioned below.

S. NO.	REAGENTS	FINAL CONC.	VOLUME (ml)
1	Tryptone	1%	1000ml
2	Yeast Extract	0.5%	
3	Sodium chloride	0.5%	
4	Agar	1.5%	

Dissolve 10g of Tryptone, 5g of yeast extract, 5g of NaCl, and 15g of Agar (if preparing agar plates) in minimum volume of distilled water and adjust the pH 7.2 with 1NNaOH/HCL. After adjusting the pH, extend the volume to 1liter in distilled water. Liquid media was transferred into flasks and covered with a cotton plug. Be sure not to fill flasks more than ¾ full. Medium was steam sterilized for 20-30 minutes in vertical autoclave. After sterilization media was allowed to cool up to ~50°C. Sterilized medium was poured in to Petri plates (Tarson Pvt Ltd) in a laminar-flow hood to maintain proper sterilization. The air bubbles appeared into plates during pouring was removed using sterilized hot inoculation loop. The media containing plates were stored at 4°C in inverted position.

CULTURING OF MICROBES ON MEDIUM

The spread plate method was employed for bacterial-cell enumeration and isolation. In the spread-plate method of addition of cells to solid medium, a small volume of culture was dropped onto the surface of agar that has already hardened in a Petri dish. To screen rhizospheric microbes, initially the loosely attached soil to the root was removed carefully in sterilized condition followed by surgical removal of roots and suspended into sterilized distilled water. The suspended sample was vortexed vigorously to detach the root bound microbes into suspension. The suspension was used for culturing of microbes. A 50µl volume of suspension was spread around the agar surface and plates were incubated at 37°C till the visible colonies appear in medium. This technique is advantageous particularly when the cells are sensitive to exposure to relatively high temperatures. This method does not require a prior melting of the solid medium.

SINGLE COLONY PURIFICATION OF MICROBES

To purify the microbial isolate from other microbial colonies, single cell streaking method was adopted. A microbial culture from a colony was picked by touching sterile loop over the colony. The loop is then used to streak the microbial cell on plate in first quarter for streaking to next quarter, the loop are again sterilized by heating & then few streak of previous quarter was touched and a new quarter was streaked. This process was continued till whole plate was streaked. After streaking the plate was incubated at 37°C till single colony appear on plate. This process was repeated many times to isolate the contamination free homogenous microbial colony.

SCREENING OF MICROBES FOR CELLULASE PRODUCTION

To screen the microbial isolate for cellulase production, pure single colony was grown on a LB media supplemented with 1.0 % Cellulose for 48 hrs at 37°C temp. After the incubation the plates were washed with sterile saline solution to remove microbial growth on medium for effective screening. These washed plates were flooded with iodine solution and plates were incubated for 5 minute at Room temperature. After the incubation the plates were washed with distilled water and zone of clearance (Halo) around the microbial growth. Presence of zone of clearance around microbial isolate indicated the bio catalytic catabolism of celluolose into glucose monomers which will inhibit the staining caused by iodine solution and lead to formation of clear zone. Microbial isolate forming zone of clearance were considered positive for cellulase production. The diameter of zone of clearance will calculated (mm). The true cellulase production efficiency of microbial isolate was confirmed by repeating the experiment thrice.

RESULT AND DISCUSSION

ISOLATION OF MICROBES FROM SOIL SAMPLE / RHIZOSPHERE

Many microbial colonies were identified after incubating the LB Agar plated layered with wheat root /soil.

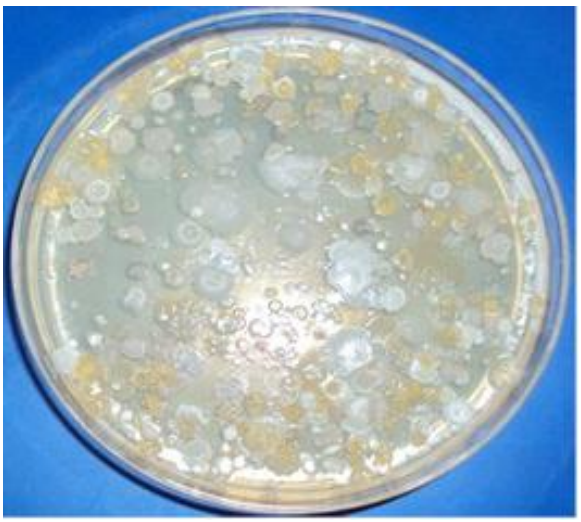

Fig. 4.1: Different types of Microbial colony.

It has been observed that rhizosphere and soil comprises of large number of different type of microbes. Which will lead to different colonies on the growth medium (Fig. 4.1). However growths of micro-organisms are slightly biased with the growth medium. As here for the growth a specific growth medium/conditions was provided which will allow only

those microbes to grown who are having similar growth requirements. To purify these microbes, initially they were picked in individually and grown onto LB medium in the form of patch (Fig. 4.2).

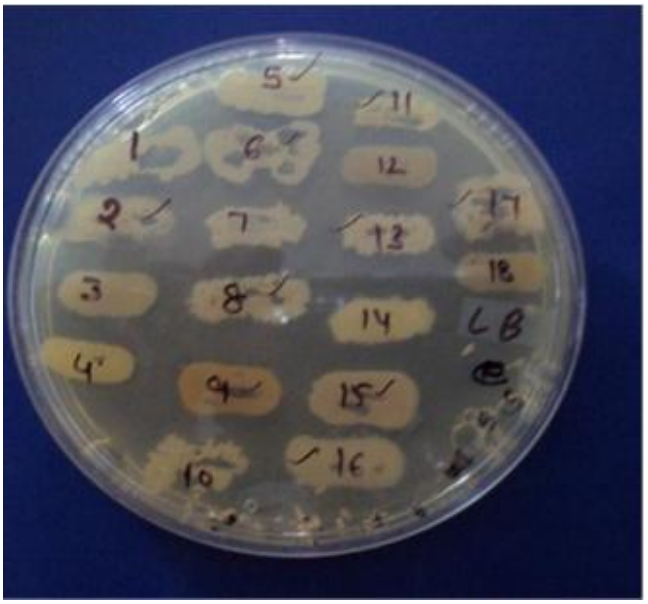

Fig. 4.2: Patched growth of microbial colonies.

These microbial cells were further purified into single colony using dilution streaking method (**Fig.4.3.).** These purified colonies were later used to screen the cellulolytic activity.

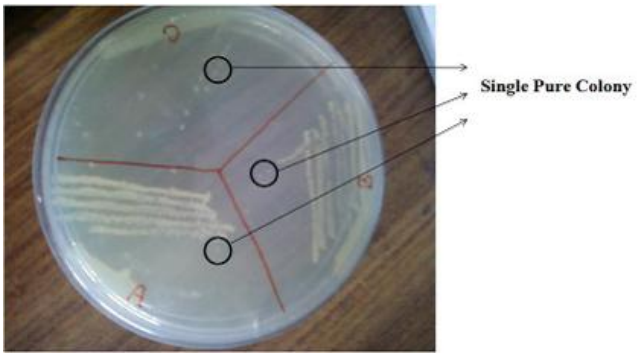

Fig. 4.3: Purified microbial colony using dilution streaking technology.

The microbial colonies were screened for cellolytic activity by growing these microbes on the LB medium supplemented with cellulose (1.0%). After the incubation these plates were stained with iodine solution and the cellulose positive microbes were checked by analyzing halo formation around the microbial growth **(Fig 4.4).**

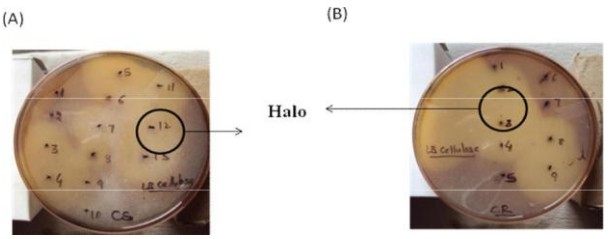

Fig 4.4: Celluolytic activity of the microorganisms isolated from the *Triticum aestivum* cultivar rhizosphere collected from canal soil (A) and Canal root Soil (B).

Activity of cellulase enzyme was analyzed in the form of size of halo formation (in mm) Table 4.1.

(A)			(B)		
S. No. Microbes	Cellulolytic Activity	Size of Halo (mm)	S.No. Microbes	Cellulolytic Activity	Size of Halo (mm)
CS1	-ve	-	CR1	-ve	-
CS2	-ve	-	CR2	+ve	13
CS3	+ve	12	CR3	+ve	19
CS4	+ve	12	CR4	+ve	8
CS5	+ve	17	CR5	-ve	-
CS6	-ve	-	CR6	-ve	-
CS7	+ve	12	CR7	-ve	-
CS8	+ve	7	CR8	+ve	11
CS9	+ve	5	CR9	+ve	18
CS10	+ve	7			
CS11	+ve	13			
CS12	+ve	14			
CS13	+ve	13			

Table 4.1: Cellulolytic activity of the microorganisms isolated from the *Triticum aestivum* cultivar rhizosphere collected from canal soil (A) and Canal root Soil (B).

The microbes with positive cellulolytic activity were matched based on texture and morphology and different looking microbial colonies were picked for the taxonomic identification.

CONCLUSION

In the present study the cellulase enzyme is isolated from the *Triticum aestivum* cultivar rhizosphere and soil. To isolate the cellulase producing microorganisms from wheat rhizosphere and soil. Initially the samples were collected under a septic condition in sterile bag (Himedia Pvt. Ltd., India) followed by culturing of microorganism onto LB growth medium. These microbial cultures were screened for cellulase production using cellulase as substrate in the growth medium followed by iodine

staining Total 15 isolates were identified for cellulase production. In which 10 isolates from the canal soil and 5 isolates from the canal root soil. These microbial isolates were checked for their identity and similarity among themselves and similarly looking microbes were discarded and only one representing strain from each group was used further.

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