ISOLATION OF CELLULASE PRODUCING BACTERIA FROM ENVIRONMENTAL SOURCES

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Abstract: Plant biomass and organic wastes generated as a result of various agronomic practices mainly comprises of organic polymer cellulose, a homopolysaccharide consisting of glucose molecules linked via β 1,4 glycosidic bonds. Cellulases are a group of enzymes which have ability to hydrolyze these complex polymers into simpler substances in an efficient manner there by decreasing the pollution load in environment. The enzyme cellulases find their application in industries and are also used in food, beverages, textiles, laundry, paper, and pulp industries etc. This study was aimed to screen the cellulolytic ability of bacteria from environmental sources and their ability to degrade cellulose.
Cellulolytic bacteria were isolated from various soil samples. Detection of extracellular cellulase production was done by way of plate assay. In this method, CIM plates were flooded with Gram's iodine giving a sharp and distinct zone around the cellulase- producing microbial colonies. It was found that forty isolates showed positive results. Highest yield of enzyme was noted at 37°C. All isolates were evaluated their cellulase activity by growing them in CIM broth. It was found that isolate ***CS16*** and ***KS18*** displayed the highest enzyme activity of ***127.77*** and ***175.92µmole/ml/min***. According to morphological and biochemical studies, the isolates were primarily identified as the *Bacillus sps.*

Keywords: Cellulose, plant biomass, organic wastes, cellulases, CIM broth, *Bacillus*

Introduction: Cellulose represents the most abundant renewable natural product in the biosphere with an estimated annual production of $4.0x10^7$ tons. The proportion of cellulose in plant tissues ranges from 20 to 45% of dry weight. Much of the cellulose in nature exists as waste paper **(Bakare *et al.,* 2005)** or as waster material from agriculture industry in the form of stalks, stems and husk **(Immanuel *et al.,* 2007).** Potential of cellulose as an alternative energy source has stimulated research into bioconversion processes which hydrolyse cellulose to soluble sugars for other industries for the production of specialty chemicals **(Immanuel *et al.,* 2007)**. Due to their vast applications and ever increasind demand, novel cellulases with better process suitability, high specific activity, and better specificity and stability are being discovered from new lineages of cellulolytic organisms.. Cellulases are consortia of free inducible enzymes which are synthesized by microorganism during their growth on cellulosic materials. The complete enzymatic hydrolysis of cellulosic materials needs different types of cellulase; endo-1,4-β-glucanase, also referred to as carboxymethyl cellulase or CMCase [EC 3.2.1.4], exo1,4-β-glucanase [EC 3.2.1.91] and β-1,4-glucosidase [EC 3.2.1.21] **(Yi J.C. 1999)**. Different microorganisms including bacteria, yeast and fungi are capable of producing cellulases **(Bhat M K 2000; Kim C 1995; Camassola *et al.,* 2004; Haakana *et al.,* 2004; Bischoff *et al.,* 2006).** This project aims to isolate cellulase producers from different environmental sources with potential applied importance.

Materials and Methods:

Sample Collection: Various soil samples were collected from different environmental source and precautions were taken to minimize the contamination chances.

Isolation of cellulase producing bacteria: Cellulase producing bacteria were isolated from environmental sources by serial dilutions technique. The sample dilutions of (10^{-3}-10^{-6}) were taken and inoculated onto nutrient agar plates by spread plate technique. The purity of culture was checked with gram staining procedure and the isolates were preserved at 4^{o}c for further experiments.

Screening of purified cultures: The purified cultures were screened for cellulase production by spreading on CIM media. The plates were incubated at 37^{o}C for 24 hours, after incubation the plates were flooded with Gram's iodine solution for few seconds. Cellulose degradation was indicated by the formation of clear zone around bacterial colonies after Iodine treatment **(Ramesh Chand Kasana *et.al.*, 2008)**. Maximum growth and a clear, well demarcated zone of hydrolysis were observed on CIM medium when incubated at 37^{o}c for 24hrs. 40 cellulase producing colonies were identified showing zone of hydrolysis upon Grams iodine treatment.

Staining and biochemical characterization: The isolates positive for cellulase production were identified by morphological and biochemical techniques (gram's staining, endospore staining, IMVIC, starch hydrolysis, catalase, oxidase, sugar fermentation,).

Determination of Cellulolytic Activity of isolates: The production medium comprised of (Peptone 5.0

gm/lt, yeast extract 5.0 gm/lt, Potassium Di Hydrogen phosphate 1.0 gm/lt, Sodium chloride 5.0 gm/lt, Magnesium sulphate 0.2 gm/lt, pH-7) containing 1% cellulose. 5% of inoculum was inoculated into the labeled CIM flasks, incubated in shaker incubator at 160rpm for 96 hours at 37°c . The fermented broth was centrifuged at 5000rpm for 5 minutes at 4°c. The supernatant (crude enzyme extract) was collected carefully and stored at 4°c for enzyme assays. **(Jahir Alam Khan *et al.*, 2011)**

Estimation of Cellulase Activity of different Isolates by DNS method: The activity was assayed using a standard method **(Mandels M. and Weber J. 1969).** The activity was estimated using 1% solution of cellulose in 0.05M citrate buffer (pH 4.8) as substrate. The reaction mixture contained 1ml citrate buffer 0.5 ml of enzyme solution and 0.5 ml of enzyme solution. The reaction was carried out at 40°c for 30 min. the amount of reducing sugar released in the hydrolysis was measured by dinitrosalicylic acid method **(Miller G.L 1959)** was expressed as 1µmole of glucose liberated per ml enzyme per minute.

Results and Discussion: Out of which 40 positive for cellulolytic activity, the colonies showing greater zone of hydrolysis were selected and subjected to shake flask fermentation and the cellulase activity was found to be **127.77 µmole/ml/min** for CS16 isolate and **175.92µmole/ml/min** for KS18 isolate. Morphological and phenotypic characteristics revealed it to be as *Bacillus* species. (Table 2). Since the sole carbon source in CIM agar was cellulose the result of the test gives strong evidence that the organisms are potential cellulase producers. These studies will add upon to the information already known and probably lead to the determination of an effective method for cost efficient process for converting cellulosic waste to bioenergy and allied aspects useful for mankind and to generate a sustainable environment.

TABLE 2

IDENTIFICATION TEST	**RESULTS**	
	16th isolate	**18th isolate**
Morphology and Gram staining	Gram positive	Gram positive
Shape	Slender rods	Slender rods
Spore	Spore forming	Spore forming
Biochemical Test:		
Indole Test	-	-
Methyl red Test	+	-
Voges Proskauer Test	-	+
Citrate utilization Test	+	+
Starch hydrolysis	+	+
Oxidase Test	+	+
Catalase Test	+	+
Nitrate reduction Test	+	+
Sugar Fermentation Test	+	+
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Oxidase Test	+	+
Catalase Test	+	+
Nitrate reduction Test	+	+
Sugar Fermentation Test	+	+

Conclusion: In view of studies conducted on cellulases, it is evident that Bacillus species are efficient degraders of cellulose. The abundant availability of cellulosic wastes can be used as a substitute as low cost carbon source for the production of industrial cellulases, which will significantly thereby reduce the production cost and also help out in reducing the pollution load.

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