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Isolation and Screening of Cellulose Degrading Microbes from Nagpur Region Soil

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ABSTRACT

Cellulose is world's most abundant organic substance and comprises a major storage form of glucose. Microbial cellulose utilization is responsible for one of the largest material flow in the biosphere therefore the aim of the study is to isolate cellulose degrading microbes from soil samples collected from different regions and to identify cellulose degrading microbes including bacteria and fungi. Two different types of cellulose-degrading bacteria and two types of cellulose degrading fungi were isolated from six different soil samples for cellulose degradation. A total of two isolates each of Thermoactinomycetes spp. and Pseudomonas spp. were isolated as well as two isolates of Aspergillus spp. and one isolate of Penicillium spp. were also isolated. Clear zone around the colony was the indication of the cellulose degradation activity of the microorganisms.

KEYWORDS

Cellulose Degradation, Bacteria, Fungi, Soil

INTRODUCTION

Cellulose is a linear polysaccharide of glucose residues with β-1, 4-glycosidic linkages. Abundant availability of cellulose makes it an attractive raw material for producing many industrially important commodity products. Sadly, much of the cellulosic waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon. With the help of cellulolytic system, cellulose can be converted to glucose which is a multiutility product, in a much cheaper and biologically favourable process. Cellulolysis is basically the biological process controlled and processed by the enzymes of cellulase system. Cellulase enzyme system comprises three classes of soluble extracellular enzymes: 1, 4-β-endoglucanase, 1, 4-β-exoglucanase, and β-glucosidase (β-D-glucoside glucohydrolase or cellobiase). Endoglucanase is responsible for random cleavage of β-1, 4-glycosidic bonds along a cellulose chain. Exoglucanase is necessary for cleavage of the nonreducing end of a cellulose chain and splitting of the elementary fibrils from the crystalline cellulose, and β-1, 4-glucosidase hydrolyses cellobiose and water-soluble cellodextrin to glucose (Shewale, 1982; Woodward and Wiseman, 1983). Only the synergy of the above three enzymes makes the complete cellulose hydrolysis to glucose (Ryu and Mandels, 1980; Wood, 1989; Samdhu and Bawa, 1992) or a thorough mineralization to H2O and CO2 possible.

Many microorganisms have been reported with cellulosic activities including many bacterial and fungal strains both aerobic and anaerobic. *Chaetomium, Fusarium Myrothecium, Trichoderma. Penicillium, Aspergillus* and so forth are some of the reported fungal species responsible for cellulosic biomass hydrolysation. Cellulolytic bacterial species include *Trichonympha, Clostridium, Actinomycetes, Bacteroides succinogenes, Butyrivibrio fibrisolvens, Ruminococcus albus, and Methanobrevibacter ruminantium* (Schwarz, 2001; Milala *et al*., 2005). Cellulase due to its massive applicability has been used in various industrial processes such as biofuels like bioethanol (Ekperigin, 2007; Vaithanomsat *et al*., 2009), triphasic biomethanation (Chakraborty *et al*., 2000); agricultural and plant waste management (Mswaka and Magan, 1998; Lu *et al*., 2004); chiral separation and ligand binding studies (Nutt *et al*., 1998).

Knowledge of cellulose-degrading microbial taxa is of significant importance with respect to nutrition, biodegradation, biotechnology, and the carbon-cycle, providing insights into the metabolism, physiology, and functional enzyme systems of the cellulolytic bacteria and fungi that are responsible for the largest flow of carbon in the biosphere. Microbial cellulose utilization is responsible for one of the largest material flow in the biosphere therefore the aim of the study is to isolate cellulose degrading microbes from soil samples collected from different regions and to identify cellulose degrading microbes including bacteria and fungi.

MATERIALS AND METHODS

Collection of Sample: Soil samples were collected from different regions in Nagpur like Sonegao (West), Koradi, Wardha, Hingna (West), Sonegao (Airport) and Kamthi.

Isolation of Bacteria:

Cellulolytic bacterial strains were isolated from soil by using serial dilutions and pour plate technique. The medium used for isolation of cellulolytic bacteria contains 1.0 % peptone, 1.0 % carboxymethylcellulose (CMC), 0.2 % K2HPO4, 1 % agar, 0.03 % MgSO4.7H2O, 0.25 % (NH4)2SO4 and 0.2 % gelatin at pH 7 for 48 hours of incubation at 30˚C. Bacterial colonies were purified by repeated streaking. The purified colonies were preserved at 4^{o}C for further identification and screening for cellulose degrading bacteria (Yin *et al*., 2010; McDonald *et al*., 2012).

Isolation of Fungi:

The Fungi were isolated by Serial Dilution Method (Tendulkar et al., 2007) and 1 ml were plated onto the potato dextrose agar plates. The plates were incubated for 7-8 days at 25-30^{0} C. Different types of fungi were isolated. These isolated fungi were then subcultured on sterile Czapek Dox agar plates. (Kadarmoidheen *et al*., 2012; McDonald *et al*., 2012).

Screening of Bacteria and Fungi for cellulolytic activity:

Screening of Bacteria: Pure cultures of bacterial isolates were individually transferred in CMC agar plates. After incubation for 48 hours, CMC agar plates were flooded with 1 % congo red and allowed to stand for 15 min at room temperature. One molar NaCl was thoroughly used for counterstaining the plates. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis (Andro *et al*., 1884).

Screening of Fungi:

Isolated fungi were placed on Czapek Dox agar medium supplemented with carboxymethyl cellulose (1.2% w/v). After an appropriate incubation period of 5 days cellulolytic activity was detected by appearance of clear zone around the colonies. Hydrolytic zones around the growing colonies were recorded for carboxymethyl cellulose activity. To enhance the visibility of hydrolytic zones, the plates were treated as follows: The plates were first flooded with 10 ml Congo red solution. Pouring off the Congo red solution, after 20 min and reflooding the plates with 10 ml of 5 ml/ liter NaCl solution for termination of colorations. After an additional 20 min, the salt solution was discarded and carboxymethyl cellulase activity was revealed by the presence of clearing zone around colonies (Mandels and Weber, 1969).

Identification of Cellulose Degrading Bacteria and Fungi: Identification of cellulolytic bacteria was carried out by method as described by Cowan and Steel (1993), Cullimore (2000) which was based on morphological and biochemical tests. All cellulose degrading fungi were identified according to Klich(2002).

RESULTS

A total of 4 bacteria and 3 fungi were isolated in these six different soil samples of Nagpur region. Out of 6 samples, 4 samples showed the presence of cellulose degrading bacteria. In this way cellulose degrading bacteria were isolated from these six positive samples and bacteria were identified as two species of *Thermoactinomycetes sp*. and two species of *Pseudomonas* spp. (Yin *et al*., 2010) (Table 1). These results were correlated with that of Chen *et al*., 2011. All the three isolated fungi were screened for their cellulolytic activity by observing the clearing zone on the Czapek Dox Agar supplemented with CMC (Carboxy Methyl Cellulose). Clear zone was not observed in both the species of *Aspergillus* spp. but was observed in case of *Penicillium* spp (Table 2). These results were similar in the context of cellulolytic activity with that of the work of Bagnara *et al*., 1985; Gupta *et al*., 2012; Ghanbary *et al*., 2010.

The bacterial and fungal isolates showed a potential to degrade cellulose which is interpreted by clear zone around microbial colonies, thus indigenous microbes could be a potential source of cellulolytic microbes which can be explored for use in many applications like feed stock for production of valuable organic compounds; for example in the present study this has been demonstrated by utilization of cellulose by producing extracellular cellulose.

.Table 1: Ethno-medicinal observations from villages of Satara District (M.S.) India

Isolates	Gram staining	Shape	Carbohydrate fermentation			TSI			Catalase	Name of Organism
			Glucose	Lactose	Mannitol	Slant	Butt	H_2S		
1	Gram Positive	Cocci	A	A	A	-	A	-	+	*Thermoactinomycetes* spp. 1
2	Gram Negative	Short rods	AG	AG	AG	-	AG	-	+	*Pseudomonas* spp.1
3	Gram Positive	Rods	AG	AG	AG	-	AG	-	+	*Thermoactinomycetes* spp.2
4	Gram Negative	Rods	AG	AG	AG	AG	AG	-	+	*Pseudomonas* spp.2

Table 2: Identification of Cellulose Degrading Fungi

Isolates	Cultural characteristics	Morphological characters	Name of Organism
1	White colonies become greenish as culture matures	Single- celled spores (conidia) in chains developing at the end of sterigma arising from the terminal bulb of the conidiophores, the vesicle; long conidiophores arise from a septate mycelium.	*Aspergillus* spp.
2	Yellow colonies	Single- celled spores (conidia) in chains developing at the end of sterigma arising from the terminal bulb of the conidiophores , the vesicle ; long conidiophores arise from a septate mycelium	*Penicillium* spp.
3	White colonies become greenish orange	Single- celled spores (conidia) in chains developed at the end of sterigma arising from the metula of the conidiophores; branching conidiophores arise from a septate mycelium.	*Aspergillus* spp.

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