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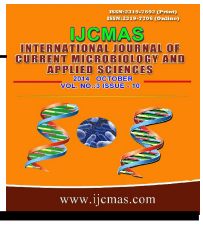

Original Research Article

Isolation, Screening and Characterization of Cellulolytic bacteria from forest soil sample

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A B S T R A C T

Keywords

Forest soil sample, physico-chemical, Biological, cellulolytic Bacteria

This study focuses on the isolation of efficient cellulolytic bacteria found in forest soil rich with plant residues .The test soil with plant residues was assessed for Physico-chemical & Biological properties in the present study. The presence of plant residues in the soil caused changes in Physico-chemical &biological properties of soil. These changes include increase in clay and silt percentages, Electrical conductivity, Water Holding capacity, Organic matter, total N, available P, K in test over control sample. However, there was a less sand, higher bacterial and fungal populations were recorded in test soil. Also, cellulose degrading bacteria were isolated from the forest soil rich with plant residues b cellulose Congo Red Agar Medium. The isolates were characterized by morphological and biochemical characters. Pure isolates were screened for cellulase activity.

Introduction

Cellulose is the most abundant biomass on the earth. Cellulases are inducible enzymes which are synthesized by large number of microorganisms either cell-bound or extra cellular during their growth on cellulosic materials (Lee and Koo, 2001). Cellulose, a crystalline polymer of D-glucose residues connected by β-1, 4 glucosidic linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature (Saha *et al.,* 2006).

Therefore, it has become considerable economic interest to develop processes for effective treatment and utilization of cellulosic wastes as inexpensive carbon sources. Complete enzymatic hydrolysis of enzyme requires synergistic action of 3 types of enzymes, namely cellobiohydrolase, endoglucanase or carboxy methyl cellulose (CMCase) and α-glucosidases (Bhat, 2000). Cellulose is commonly degraded by an enzyme called

cellulase. Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyse the cellulolysis (or hydrolysis) of cellulose (Immanuel *et al.,* 2006). This enzyme is produced by several microorganisms, commonly by bacteria and fungi. Cost of cellulase in enzymatic hydrolysis is regarded as a major factor (Kanokphorn *et al.,* 2011). Cellulases have attracted much interest because of the diversity of their application. The major industrial applications of cellulases are in textile industry for 'bio-polishing' of fabrics and producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness (Kanokphorn, 1998). Application of enzymes in textile, food, detergent, leather and paper industries demands identification of highly stable enzymes active at extreme pH and temperature (Abdelnasser and Ahmed, 2007). Cellulase is used in the fermentation of biomass into biofuels (Cherry and Fidantsef, 2003), fibre modification and they are even used for pharmaceutical applications. Bacteria has high growth rate as compared to fungi has good potential to be used in cellulose production. Celluloytic property of some bacterial genera such as Cellulomonas species, Pseudomonas species, Bacillus species and Micrococcus species were reported (Nakamura and Kappamura, 1982). Enzyme production is closely controlled in microorganisms and for improving its productivity these controls can be ameliorated. Cellulase yields appear to depend on a complex relationship involving a variety of factors like inoculum size, pH, temperature, presence of inducers, medium additives, aeration, and growth time (Robson and Chambliss, 1989).The aim of this study was to isolation, screening and characterization of cellulase producing bacteria from forest soil.

Materials and Methods

Collection of soil samples:

Soil samples were collected from surrounding areas of Nallamala forest Srisailam, Kurnool district of Andhrapradesh India (Test soil). Soil sample without plant residues served as control was collected from adjacent site of forest. Soil samples both test and control were used for determination of Physico-chemical and biological properities. These two soil samples were air dried and mixed thoroughly to increase homogeneity and shifted to <2 mm sieves for determination of soil texture.

Physico-Chemical Properties of soil

The physico-chemical and biological properties of test and control soils were determined by the following standard procedures. The soil particles like sand, silt and clay contents were analyzed with the use of different sieves by the method of Alexander (1961). Whereas water holding capacity, organic carbon, total nitrogen, and soluble phosphorous of soil samples were determined by the methods of a Johnson and Ulrich (1960), Walkey and black, (1934), Microkjeldhal (Johnson and Ulrich, 1960) and Kuprevich and Shcherbakova (1972), respectively. Electrical conductivity and pH were determined by Elico conductivity meter and pH meters, respectively.

Biological parameters

Microflora such as bacterial and fungal populations of both test and control soil samples were enumerated by serial dilution technique. One gram of each soil sample was serially diluted and 0.1ml was spreaded with a sterile spreader on Nutrient agar

medium (pH7.2) and Czapeck- Dox medium for the growth of bacteria and fungi respectively. Nutrient agar plates were incubated at 37^{o} C for 24 hr, whereas Czapeck-Dox plates were at room temperature for 7 days. After incubation period, colonies formed on the surface of the medium were counted by colony counter (Alexander, 1961).

Isolation of cellulolytic bacteria

Ten grams of soil sample was suspended in 90 ml sterile distilled water and serial dilutions were prepared by transferring 1ml of diluted suspension to 9ml of sterile distilled blanks. A 0.1ml suspension of 10^{-4} and 10^{-5} dilution were plated on the modified cellulose Congo red agar (CCRA) medium (Hendricks *et al.,* 1995) and incubated for 5 days at 28±2°C.For bacterial isolates candid antibiotic (100µg/ml medium) was added to the CCRA medium after autoclaving to prevent the fungal growth.

Screening of cellulolytic bacteria

The Colonies grown on CCRA media were not considered as pure even though only one type of colony appeared and exhibited the zone of clearing. These bacterial colonies were purified by taking single colony each time in a streak plate method on cellulose-Congo red agar medium repeatedly at least seven times until plate contained uniform one type of colonies. The purified colonies were checked for cellulolytic activity by the method described (Teather *et al.,* 1982). In this method, the bacterial colonies grown on cellulosic medium without Congo red for two days at 28±2°C, then the medium was flooded with an aqueous solution of Congo red (1mg/ml) for 15 min to allow to reach to cellulose. The excess Congo red solution was then poured off, and plates were further flooded with 1M NaCl for 15 min. The cellulose hydrolysis zones were visualized. The plates were further stabilized at least for 2 weeks by flooding with 1M HCl, which changes the dye colour red into blue and inhibited further enzyme activity. The bacterial isolates were maintained on nutrient agar plates for routine use and stored at 4°C.

Identification of cellulolytic bacteria

Identification of cellulolytic bacteria was carried out by method as described by Cowen and Steel (1993) and Cullimore (2000) which was based on morphological and biochemical tests.

Results and Discussion

The analytical results of Physico-chemical and biological parameters of both the test and control soils were presented in Table 2. Test soil samples underwent changes in all the measured parameters of Physico-chemical and biological parameters in comparison to control soils. The soil texture in terms of percentages of clay, silt and sand were 46, 36, 18 and 50, 40 and 10 in test and control soil, respectively as shown in Table 2. The above results indicated that soils with plant residues had relatively lower sand content and higher clay and silt contents than control soil. This may be due to the micronutrients discharged into the soil through plant wastes reduce the porosity of the soil resulting in poor yields. More recently, similar results were also noticed by Pradeep and Narasimha (2012) in soil polluted with leather industry effluents caused drop in sand content and hike in clay content. Surprisingly, in their observation, sand content was adversely affected with increasing the quantity of effluent in soil.

Table.1 Physico-Chemical Characteristics of Test & Control soil samples

Character	**Control** [b]	**Test** [a]
Color	Black	Thick black
Odor	Normal	Bad
pH [1:1.25 soil–water slurry]	7.0	9.0
Texture: Clay [%] Silt [%] Sand [%]	 46 36 18	 50 40 10
Electrical conductivity [µmhos/cm]	0.24	0.68
60%Water-holding capacity [mL g−1]	0.8	1.4
Organic matter [%]	2.8	8.0
Total nitrogen [g kg−1 soil]	2.0	9.0
Available phosphorus [P2O5] in [kg/ha]	198	400
Available potassium [K2O] in [kg/ha]	800	1580

a. soil polluted with plant residues (Test)
b.soil without polluted plant residues(Control)

Table.2 Biological Parameters of Test & Control soil samples

Micro flora	**Control** [b]	**Test** [a]
Bacteria	30×10^7	68×10^7 [c]
Fungi	20×10^5	38×10^5

a. soil polluted with plant residues (Test)
b.soil without polluted plant residues(Control)
c.Colony forming units per gram of soil.

Table.3 Morphological Characteristics of Bacteria

S.No	**Characters Tested**	**Isolate-1**	**Isolate-2**
1	Colony Morphology	Whitish round non slime	Whitish round with slime
2	Cell Morphology	Cocco Bacilli	Rod
3	Gram's Stain	Positive	Negative
4	KOH Test	Negative	Negative
5	Spore Stain	Positive	Negative
6	Sporulation	Positive	Negative
7	Motility Test	Positive	Negative

Table.4 Biochemical Characteristics of Bacteria

S.No	Name Of The Test	Isolate-1	Isolate-2
1	Amylase Production	+	+
2	Gelatin Hydrolysis	+	+
3	Catalase hydrolysis	+	+
4	Nitrate Reduction Test	+	+
5	Indole Test	-	-
6	Methyl Red Test	-	-
7	V-P Test	+	+
8	Simmons citrate utilization Test	+	+
9	Carbohydrate fermentation Test	-	-
10	Urease Test	-	-
11	Starch Hydrolysis	+	+
12	Casein Hydrolysis	+	+
13	H-L Test	+	+
14	Lipase Activity	+	+

Also, Nizamuddin et al., (2008) reported that discharge of dairy factory effluents decreased the soil sand content. Numerous results reported that the soils treated with long term sewage effluents (Abdelnainm *et al.,* 1987), effluents of cotton ginning mills (Narasimha *et al.,* 1999) and sugar industry (Nagaraju *et al.,* 2007) increased the clay content of soil and subsequently improved the soil texture and fertility.

Higher water holding capacity and electrical conductivity were observed in test soil than control (Table 2) may be due to accumulation of organic wastes and salts in plant residues. Likewise, soil discharged with effluents from cotton ginning mills (Narasimha *et al.,* 1999), paper mills (Medhi *et al.,* 2005), and sugar industry (Nagaraju *et al.,* 2007) increased the water holding capacity and electrical conductivity. In contrast, soils polluted with cement industries had low water holding capacity and higher electrical conductivity (Shanthi, 1993; Sivakumar *et al.,* 1995). Surprisingly, there was no observable change in soil pH upon discharging of plant wastes. This could be due to neutral nature of plant residues. Furthermore, half of the higher content of organic matter was recorded in test soil over control. The contents of total nitrogen and phosphorous in soils with plant residues were generously higher in test soil than control soil (Table 2). Kannan and Oblisami (1990) observed that irrigation of sugarcane crops with combined pulp and paper mill effluent increased soil pH, organic C, N, P, and K. A perceptible change in bulk density, pH, EC, OC was observed under continuous paper mill effluent irrigation.

Similarly, discharge of effluents from cotton ginning mills (Narasimha *et al.,* 1999) and sugar industry (Nagaraju *et al.,* 2007) increased the total nitrogen and phosphorous contents compare to the

control soil. Furthermore, micro flora of both soil samples were enumerated and listed in the Table 3. One fold higher bacterial and fungal population noticed in test soil over control sample. Subsequently, two bacteria were isolated from the test soil by enrichment method and morphological and biochemical characteristics of the two cultures were analyzed. One isolate (Isolate 1) was a gram positive, cocco bacilli and spore former (Table 4), while another one (Isolate 2) was gram negative, rod shape, and non spore forming bacterium. Furthermore, isolate 1 has shown positive reaction in casein hydrolysis, Catalase, H-L test, lipase and citrate utilization (Table 5). Whereas isolate 2 has shown positive result in the hydrolysis of starch, gelatin, and casein. Additionally, it has shown positive reaction in Catalase, H-L, lipase, amylase, nitrate reduction and citrate utilization. Based on morphological and Biochemical characters, those two isolates resembles *Bacillus* and *Pseudomonas sp.* respectively.

References

Abdelnainm, E.M., Rao, M.S., Wally, T.M., Nashar, E.M.B. 1987. Effect of prolonged sweage irrigation on some physical properties of sandy soil. *Biol. Wastes,* 22: 269–274.

Abdelnasser, S.S.I., Ahmed, I.E. 2007. Isolation and identification of new cellulases producing thermophilic bacteria from an Egyptian hot spring and some properties of the crude enzyme. *Aust. J. Basic Appl. Sci.*, 1(4): 473–478.

Alexander, M. 1961. Introduction to soil microbiology. Wiley Estern Ltd. New Delhi.

Bhat, M.K. 2000 Cellulases and related enzymesin biotechnology. *Biotech. Adv.,* 1: 355–383.

Cavaco-Paulo, A. 1998. Mechanism of cellulose action in textile processes. *Carbohydr. Polym.,* 37: 273–277.

Cherry, J.R., Fidantsef, A.L. 2003 Directed evolution of industrial enzymes: an update. *Curr. Opin. Biotechnol.,*14: 438–443.

Cowan, S.T., Steel, K.J. 1993. Manual for the identification of medical bacteria, 3rd edn., Cambridge University press, USA. Pp.150–152.

Cullimore, D.R. 2000. Practical Atlas for bacterial Identification. Lewis publishers, Boca Roton, London, New York. 209 Pp.

Hendricks, Charles W., Doyle, Jack D., Bonnie, H. 1995. A new solid medium for enumerating cellulose utilizing bacteria in soil. *Appl. Env. Microbial.*, 61: 2016–2019.

Immanuel, G., Dhanusha, R., Prema, P., Palavesam, A. 2006 Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment *Int. J. Environ. Sci. Technol.,* 3: 25–34.

Jackson, M.L. 1973. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi. 498 Pp.

Johnson, C.M., Ulrich, A. 1960. Determination of moisture in plant tissues. Calif. Agri. Bull. In: S.A.Wilde *et al.,* (Ed.), Soil and plant analysis for tree culture Obortage publishing Co., Oxford and Bombay.

Kannan, K., Oblisami, G. 1990. Influence of irrigation with pulp and paper mill effluent on soil chemical and microbiological properties. *Biol. Fertil. Soils,* 10: 197–201.

Kanokphorn, S., Piyaporn, V., Siripa, J. 2011. Isolation of novel cellulase from agricultural soil andapplication for ethanol production. *Int. J. Adv. Biotechnol Res.,* 2(2): 230–239.

Kuprevich, V.E., Shecherbakova T.A. 1972. Comparative enzymatic activity in diverse types of soil organisms. In: P.C. Mishra (Ed.), Soil Pollution and Soil Organisms. Ashsih Publishing House, New Delhi.

Lee, S.M., Koo, M.Y. 2001 Pilot-scale production of cellulose using *Trichoderma reesei*Rut C-30 infed-batch mode. *J. Microbiol. Biotechnol.,* 11: 229–233.

Medhi, U.J., Talukdar, A.K., Deka, S. 2005. Physicochemical characteristics of lime sludge waste of paper mill and its impact on growth and production of rice. *J. Ind. Pollut.. Control,* 21: 51–58.

Nagaraju, M., Narasimha, G., Rangaswamy, V. 20007. Impact of effluents of sugarcane industry on Physicochemical and biological properties. *J. Ind. Pollut Control,* 23: 73–76.

Nakamura, K., Kappamura, K. 1982. Isolation and identification of crystalline cellulose hydrolyzing bacterium and its enzymatic properties. *J. Ferment. Technol.,* 60 (4): 343–348.

Narasimha, G., Babu, G.V.A.K., Rajashekar Reddy, B. 1999. Physico-chemical and biological properties of soil samples collected from soil contaminated with effluents of cotton ginning industry. *J. Environ. Biol.,* 20: 235–239.

Nizamuddin, S., Sridevi, A., Narasimha, G. 2008. Impact of dairy factory effluents soil enzyme activities. *Ecol. Environ. Conserv.,* 14: 89–94.

Pradeep, M.R., Narasimh. G. 2012. Effect of leather industry effluents on soil microbial and protease activities. *J. Environ. Biol.,* 33: 39–42.

Robson, L.M., Chambliss, G.H. 1989. Characterization of the cellulolytic activity of a Bacillus isolate. *Appl. Environ. Microbiol.,* 47: 1039–1046.

Saha, S., Roy, R., Sen, S.K., Ray, A.K. 2006. Characterization of cellulase-producing bacteria from the digestive tract of tilapia, *Oreochromismossambica* (Peters) and grass carp, *Ctenopharyn godonidella* (Valenciennes). *Aquaculture Res.,* 37: 380–388.

Shanthi, M. 1993. Soil biochemical process in industrially polluted area of cement industry. M.Phil dissertation, Sri Krishnadevaraya University, Anantapur, A.P., India.

Sivakumar, S., John de Brito, A. 1995. Effect of cement pollution on soil fertility. *J. Ecotoxicol. Environ. Monit.,* 5: 147–149.

Teather, Ronald M., Wood, Peter J. 1982. Use of congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Appl. Env. Microbial*., 43: 777–780.

Walkley, A., Black, I.A. 1934. An examination of theDegtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.,* 63: 251–263.