

Isolation and Screening of Cellulose Degrading Microbes from Nagpur Region Soil

Gomashe AV, Gulhane PA and Bezalwar PM*

Department of Microbiology, S.S.E.S.A's Science College, Congress Nagar, Nagpur-440012 (MS), India

*Corresponding author email: pratikmbezalwar@gmail.com

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 *Thermoactinomyces* 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 hydrolysis. Cellulolytic bacterial species include *Trichonympha*, *Clostridium*, *Actinomyces*, *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 Sonogao (West), Koradi, Wardha, Hingna (West), Sonogao (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°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°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 *Thermoactinomyces 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 ₂ S | | |
| 1 | Gram Positive | Cocci | A | A | A | - | A | - | + | <i>Thermoactinomyces spp. 1</i> |
| 2 | Gram Negative | Short rods | AG | AG | AG | - | AG | - | + | <i>Pseudomonas spp.1</i> |
| 3 | Gram Positive | Rods | AG | AG | AG | - | AG | - | + | <i>Thermoactinomyces spp.2</i> |
| 4 | Gram Negative | Rods | AG | AG | AG | AG | AG | - | + | <i>Pseudomonas spp.2</i> |

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. | <i>Aspergillus</i> 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 | <i>Penicillium</i> 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. | <i>Aspergillus</i> spp. |

REFERENCES

- Andro T, Chambost JP, Kotoujansky A, Cattano J, Barras F (1984) Mutants of *Erwinia chrysanthemi* defective in secretion of pectinase and cellulase. *J Bacteriol.*, 160:1199-1203.
- Bagnara C, Toci R, Gaudin C, Bélaïch JP (1985) Isolation and characterization of a cellulolytic microorganism, *Cellulomonas fermentans*, sp. nov. *Int. J. Syst. Bacteriol.*, 35:502-507.
- Chakraborty N, Sarkar GM, Lahiri SC (2000) Cellulose degrading capabilities of cellulolytic bacteria isolated from the intestinal fluids of the silver cricket. *Environmentalist*, 20(1): 9-11.
- Chen HJ, Chang HJ, Fan C, Chen WH, Lee MS (2011) Screening, isolation and characterization of cellulose biotransformation bacteria from specific soils. *International Conference on Environment and Industrial Innovation*, 12: 216-220.
- Cowan ST, Steel KJ (1993) Manual for the identification of medical bacteria 3rd edn. Cambridge University press, USA, pp: 150-152.
- Cullimore DR (2000) Practical Atlas for Bacterial Identification. Lewis Publishers, Boca Raton, London, New York, pp: 209.
- Ekperigin MM (2007) Preliminary studies of cellulase production by *Acinetobacter anitratus* and *Branhamella* spp. *African Journal of Biotechnology*, 6(1): 28-33.
- Ghanbary MAT, Lotfi A, Asgharzadeh A, Telmadarrehei T, Javadi MA (2010) Laboratory simulation of cellulomonas degradation by soil *Aspergillus*. *American Eurasian J. Agric. & Environ. Sci.*, 7(2): 146-148.
- Gupta P, Samant K, Sahu A (2012) Isolation of cellulose-degrading bacteria and determination of
- Kadarmoidheen M, Saranraj P, Stella D (2012) Effect of cellulolytic fungi on the degradation of cellulosic agricultural wastes. *International Journal of Applied Microbiology Science*, 1(2): 13-23.
- Klich MA (2002) Identification of common *Aspergillus* species. CBS Netherlands, pp: 116.
- Lu WJ, Wang HT, Nie YF (2004) Effect of inoculating flower stalks and vegetable waste with ligno-cellulolytic microorganisms on the composting process. *Journal of Environmental Science and Health, Part B*, 39(5-6): 871-887.
- Mandels M, Weber J (1969) Production of cellulases. *Adv. Chem. Ser.*, 95: 391- 414.
- McDonald JE, Rooks DJ, McCarthy AJ (2012) Methods for the isolation of cellulose-degrading microorganisms. *Methods Enzymol.*, 510: 349-374.
- Milala MA, Shugaba A, Gidado A, Ene AC, Wafar JA (2005) Studies on the use of agricultural wastes for cellulase enzyme production by *A. niger*. *Journal of Agriculture and Biological Science*, 1: 325-328.
- Mswaka AY, Magan N (1998) Wood degradation and cellulase and ligninase production, by *Trametes* and other wood-inhabiting basidiomycetes from indigenous forests of Zimbabwe. *Mycological Research*, 102(11): 1399-1404.
- Nutt A, Sild V, Prttersson G, Johansson G (1998) Progress curve as a means for functional classification of cellulases. *European Journal of Biochemistry*, 258:200.
- Ryu DDY, Mandels M (1980) Cellulases: biosynthesis and applications. *Enzyme and Microbial Technology*, 2(2):91-102.
- Samdhu DK and Bawa S (1992) Improvement of cellulase activity in *Trichoderma*. *Applied Biochemistry and Biotechnology*, 34-35(1):175-192.
- Schwarz WH (2001) The cellulosome and cellulose degradation by anaerobic bacteria. *Applied Microbiology and Biotechnology*, 56(5-6): 634-649.
- Shewale JG (1982) Glucosidase: its role in cellulase synthesis and hydrolysis of cellulose. *International Journal of Biochemistry*, 14(6):435-443.
- Vaithanomsat P, Chuichulcherm S, Apiwatanapiwat W (2009) Bioethanol production from enzymatically saccharified sunflower stalks using steam explosion as pretreatment. *Proceedings of World Academy of Science, Engineering and Technology*, 37: 140-143.
- Wood TM (1989) Synergism between enzyme components of *Penicillium pinophilum* cellulase in solubilizing hydrogen ordered cellulose. *Journal of Biochemistry*, 260: 37-43.
- Woodward and Wiseman A (1983) Fungal and other β -d-glucosidases: their properties and applications. *Enzyme and Microbial Technology*, 4(2): 73-79.
- Yin LJ, Huang PS, Lin HH (2010) Isolation of cellulase-producing bacteria and characterization of the cellulase from the isolated bacterium *Cellulomonas* spp. YJ5. *J Agric Food Chem*, 58:9833-9837.
- Tendulkar SR,, Saikumari YK, Patel V, Raghotama S, Balaram P and Chattoo BB. (2007) Isolation, purification and characterization of an antifungalmolecule produced by *Bacillus licheniformis* BC98, and its effect on phytopathogen *Magnaporthe grisea*. *Journal of Applied Microbiology*, 103:2331-2339.

© 2013| Published by IJLSCI

Cite this article as: Jagtap DK, Patil HS, Jakhi PS (2013) Ethno-medicinal survey of some plants from villages of Khatav Tahashil (M.S.) India, *Int. J. of Life Sciences*, 1(4): 291-293.

Source of Support: Nil,

Conflict of Interest: None declared