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Isolation and screening of potential cellulolytic bacteria from coir retting effluent

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Abstract
Cellulases are the group of hydrolytic enzymes and they are capable of degrading all types of cellulosic waste materials. Present study focuses on the isolation and screening of cellulolytic bacteria from the retting effluent and its nearby estuary. Six different bacterial strains were isolated and screened for cellulase production. The cellulolytic gram positive bacterial isolate includes *Bacillus spp, Micrococcus spp* and *Staphylococcus spp* and the gram negative bacterial isolate includes *Pseudomonas spp, Klebsiella spp* and *Vibrio spp* respectively. Physicochemical characteristics of the retting effluent were also analysed. Low BOD and high COD of the effluent is due to the presence of high molecular weight compounds such as cellulose and lignin. It shows that the physicochemical characteristics were slightly apart from the optimum values that are required in safe aquatic environment to establish an aquatic ecosystem on water body. The results of this study add significant knowledge on the diversity of cellulolytic bacteria from the retting effluent for the production of industrial enzyme.

Keywords: retting effluent, cellulose, cellulolytic bacteria, physico-chemical characteristics.

1. Introduction

Cellulose is the most abundant biopolymer on earth and most dominating agricultural wastes. This cellulosic biomass is a renewable and abundant resource with great potential for bioconversion to value added bio-products. The cellulosic waste material can be hydrolyzed to glucose and other soluble sugars by using cellulase enzymes of bacteria and fungi [1]. The specific cellulolytic activity shown by the bacterial species is found to be depending on the source of occurrence [2]. Some bacterial species viz., *Cellulomonas* species, *Pseudomonas* species, *Bacillus* species and *Micrococcus* have cellulolytic property [3]. A large number of microorganisms are capable of degrading cellulose, only a few of them produces significant quantities of cell-free bioactive compounds capable of completely hydrolyzing crystalline cellulose *in vitro*. Numerous investigations have reported the degradation of cellulosic materials, but few studies have examined which microorganisms had met the industrial requirement [4]. Attempts to increase the production of cellulose enzymes from bacteria including mutation [5, 6], protoplast fission [7], optimization of medium composition and environmental factors [8] have also been made. The bioconversion of various complex cellulosic waste materials such as bagasse [9], corncob [10] and saw dust [11] have been reported. Coir dust and fibres are also major biowastes of coir industries of India, discarded along with coir retting effluent to estuarine environment [12]. The husks being lignocellulosic in nature release polyphenols into the backwaters during the process of retting. These large volumes of cellulosic waste generated are difficult to degrade and cause imbalances in the ecosystem [13]. All these processes contribute to pollution of water with highly turbid greyish water and mercaptan-like smell that pervades the yards and nearby areas. This is reported to have a devastating effect on flora and fauna [14]. Hence the present study was carried out to analyse the physico-chemical characters and to screen the cellulolytic bacteria of the retting effluent and estuarine environment.

2. Materials and methods

2.1. Collection of samples: Water samples were collected in plastic water cans (2 l capacity) from the coir retting pond and estuary situated in Rajakkamangalam, Kanyakumari district, Tamil Nadu during the month of January, 2008. Samples were collected during morning hours between 9.00 and 10.00 A.M.

2.2. Physico-chemical analysis: The water samples were washed with 20% nitric acid and subsequently with demineralized water. Then they were refrigerated at 40°C prior to analysis. Physico-chemical analysis (pH, electrical conductivity, alkalinity, phosphate, calcium hardness, iron, sodium, phosphate, magnesium hardness, total hardness, biological oxygen demand and chemical oxygen demand) was carried out according to standard methods [15].

2.3. Bacteriological analysis: The water samples collected in a sterile plastic container were serial diluted in the laboratory and the colonies developed (pour plate method) were counted and identified using biochemical tests. Biochemical tests include gram's staining, indole test, MR-VP test, voge's proskaur test, catalase test, oxidase test, simmon citrate test, TSI test, oxidase test, carbohydrate fermentation test. Depending on the results obtained the bacterial genera were identified using Bergey's Manual of Determinative Bacteriology.

2.4. Screening of Cellulolytic Bacteria:
Pure cultures of bacterial isolates were individually transferred in nutrient agar with 1% of Carboxy Methyl Cellulose in agar plates. After incubation for 48 hours, nutrient with 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 counter staining the screened plates [16]. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis. The bacterial colonies have the largest clear zone were selected for identification.

3. Results and discussion
Retting is the basic process involved in the manufacture of coir. It is a biological process which involves the pectinolytic activity of microorganisms especially bacteria and fungi present in the retting grounds that liberate large quantities of organic substances into the medium [17]. Habitats where these substrates are present are the best sources for isolation of cellulolytic microorganisms. Several microorganisms have been discovered for decades which have capacity to convert cellulose into simple sugars [18] but the need for newly isolated cellulose degrading microorganism still continues [19].

In the present study, the total viable count of water samples (retting pond 1,2 and estuary) during the month of January 2008 showed that higher number of colonies were found in 10^{-1} dilution (Table -1).They were 220, 429 and 317 CFU/ml respectively in retting pond-1, retting pond-2 and estuary. In the present study thirteen bacterial strains were isolated from the three stations and screened for cellulolytic activity. Six isolates were found to be cellulolytic bacteria. The cellulolytic gram positive bacterial isolate includes *Bacillus spp, Micrococcus spp* and *Staphylococcus spp* and the gram negative bacterial isolates includes *Pseudomonas spp, Klebsiella spp* and *Vibriospp* respectively. Researchers studying on cellulolytic activity have isolated various bacteria from different environmental sources. Aerobic cellulolytic bacteria was isolated from forest and farming soils and it was determined that they had the ability to decompose cellulose [20]. Eight cellulolytic bacterial strains were isolated from cow dung samples [21]. *Bacillus* sp, *Clostridium*, *Pseudomonas* and *Erwinia* showed optimum cellulase production [22]. Ten bacterial strains such as *Bacillus spp1, Bacillus spp2, Micrococcus spp, Staphylococcus spp, Clostridium spp, Acinetobacter spp, Pseudomonas spp1, Klebsiella spp, Proteus spp1 and Enterobacter spp,* were isolated from decayed sawdust which showed better cellulolytic activity [23]. It is concluded that agro-land industrial waste residues are generally considered the best substrate for the production of cellulose enzymes [1].

Disposal and management of ligno-cellulosic waste had become a major environmental concern worldwide [14]. In the present study. the intensity of pollution caused by retting process is highlighted. pH of the selected stations were 9.41(retting pond-1), 9.61(retting pond-2) and 8.4(estuary) respectively. The pH of the effluent was generally neutral to alkaline range [24]. The other important factors that influence the pH variations are rainfall, river discharge and exchange from sea and flow from retting grounds [25].

Total dissolved solids denoted kinds of minerals present in water. TDS is composed mainly of carbonates,bicarbonate,Cl^-,SO_4^{2-},PO_4^{2-}, -P and NO_3^{-1}-N,Ca^{++},Na^+,K^+,F^{++} and Fe^{++} [26]. In the present investigation, TDS was 3549 mg/l (retting pond-1), 2377 mg/l (retting pond-2) and 2170 mg/l (estuary). The TDS value was above the desirable range (below 500 ppm) as standardized by World Health Organization (WHO).

Electrical Conductivity (EC) in water is due to ionization of dissolved inorganic solids and becomes a measure of total dissolved solids. In the present investigation, EC was 5143 µS/cm (retting pond-1), 3445 µS/cm (retting pond-2) and 2876 µS/cm (estuary) respectively. This value was higher than WHO recommended threshold of 250 µS/cm, for effluents discharged into receiving water bodies.The higher values may be due to accumulation of ions owing to evaporation, biological turnover and interaction with sediments [27].

Chlorides in water are contributed by the salts of sodium and calcium [28]. Chloride concentration was 1700 mg/l (retting pond-1), 950 mg/l (retting pond-2), 1420 mg/l (estuary) in the selected station. Magnesium hazard is likely to develop only if the level exceeds 50 mg/l [29]. the results showed that magnesium content was 202mg/l (retting pond-2), 106mg/l(retting pond-1), 71 mg/l (estuary). Nitrate content was 12mg/l (retting pond-1), 12mg/l (retting pond-2), 8. 16 mg/l (estuary) in the selected retting pond. The nitrate level was above the WHO permissible level which is 10mg/l [30]. NO_3 in water is toxic at high concentration and has been linked to methemoglobinema in infants. [31]. Nitrate contamination in drinking water by infants can lead to oxygen deficiency in the blood, a potentially fatal condition [32].

The ammonia content recorded in the selected station were 10 mg/l (retting pond-1), 8.85 mg/l (retting pond-2), 2. 18 mg/l (estuary). Pure water rarely contains more than 0.04 mg/l of free and saline ammonia [33]. Sulphate content ranged 34 mg/l (retting pond-1), 51 mg/l (retting pond-2), 22mg/l (estuary) in the present study. It was within the WHO permissible limit (200 mg/l). The introduction of phosphorus in the form of phosphates in aquatic environment is a major cause of eutrophication. Phosphorus occurs naturally, almost solely as phosphate [34.35]. In the present study phosphate content was 2.6 mg/l (retting pond-1), 2.74 mg/l (retting pond-2) and3.40mg/l (estuary). The level of phosphate was lower than the WHO limit of 5mg/l for the discharge of wastewater into river.

The required oxygen demand expressed as BOD and COD is an important parameter for the evaluation of wastewater. The BOD may be defined as the oxygen required for the microorganism to carry out biological decomposition of dissolved solids or organic matter in the wastewater under aerobic conditions at standard [36]. In the present study, biochemical oxygen demand was 87mg/l (retting pond-1), 89.6mg/l (retting pond-2), 72 mg/l (estuary). Chemical oxygen demand (COD) test can be used to measure content of organic matter of wastewater which is useful in the control of treatment processes [36]. Chemical oxygen demand was 225.4mg/l (retting pond-1), 268.6mg/l (retting pond-2) and 210mg/l (estuary) in the selected sampling station. The high COD load could be due to the intense of retting activity and run off from the surrounding areas [24].The reason of low BOD and high COD is the presence of high molecular weight compounds such as cellulose and lignin [37]. Effluent runoff into rivers and streams can be highly lethal to aquatic life [38, 39].

Inorder to protect ecosystems in the environment, the introduction of treatment or waste reduction at source could be used in addition to the regulations in order to control levels of pollution agents being released into the environment. The results of this study add significant knowledge on the diversity of cellulolytic bacteria from the retting effluent for the production of industrial enzyme. Further investigations are required to make use of the full potential of these organisms for cellulase production by modern technology.

4. Tables

Table 1: Physico-chemical characteristics of the selected stations

Parameters	Retting pond-1	Retting pond-2	Estuary
Colour	Blackish	Blackish	Blackish brown
Odour	Disagreeable	Disagreeable	Disagreeable
TURBIDITY(NTU)	98	72	40
TDS	3549	2377	2170
E.C (µS/cm)	5143	3445	2876
p^H	9.41	9.61	8.4
MAGNESIUM (mg/l)	202	106	71
FREE AMMONIA (mg/l)	10	8.85	2.18
NITRATE (mg/l)	12	12	8.16
CHLORIDE (mg/l)	1700	950	1420
SULPHATE (mg/l)	34	51	22
PHOSPHATE (mg/l)	2.6 ± 0.1	2.74 ± 0.1	3.40 ± 0.1
BOD (mg/l)	87 ± 6.2	89.6 ± 5.81	72 ± 6.4
COD (mg/l)	225.4 ± 20.6	268.6 ± 24.32	210 ± 19.86

Table 2: Total viable count of bacteria in the selected stations

Station	Dilution		
	10^{-1}	10^{-2}	10^{-3}
Coir Retting Pond-1	220	56	13
Coir Retting Pond-2	429	126	81
Estuary	317	42	34

Table 3: Morphological characteristics of the isolated bacteria

S.NO.	Bacteria	Gram Staining	Morphology	Motility	Colour	Colony shape	Margin
1.	*Bacillus* sp.	+	Rod	+	cream	circular	undulate
2.	*Pseudomonas* sp.	-	Rod	+	cream	circular	Entire
3.	*Klebsiella sp.*	-	Rod	-	cream	circular	smooth
4.	*Vibrio* sp.	-	Rod	+	cream	circular	Entire
5.	*Staphylococcus* sp.	+	Cocci	-	cream	Irregular	Undulate
6.	*Micrococcus* sp.	+	Cocci	-	cream	circular	Entire

Table 4: Biochemical characteristics of the cellulolytic bacterial isolates

Biochemical characteristics	Bacterial isolates					
	1	**2**	**3**	**4**	**5**	**6**
Indole production	+	-	-	+	+	+
Methyl red reaction	-	-	-	-	+	+
Voges-Proskauer reaction	-	+	+	+	+	+
Citrate utilization	+	-	+	+	+	-
Catalase reaction	+	+	+	+	+	+
Oxidase reaction	-	-	-	+	+	+
Urease production	+	+	+	-	-	-
Gelatin hydrolysis	+	-	-	-	+	-
Nitrate	+	-	+	+	+	
Strain identified	*Micrococcus sp.*	*Staphylococcus sp.*	*Klebsiella sp.*	*Pseudomonas sp.*	*Bacillus sp.*	*Vibrio sp*

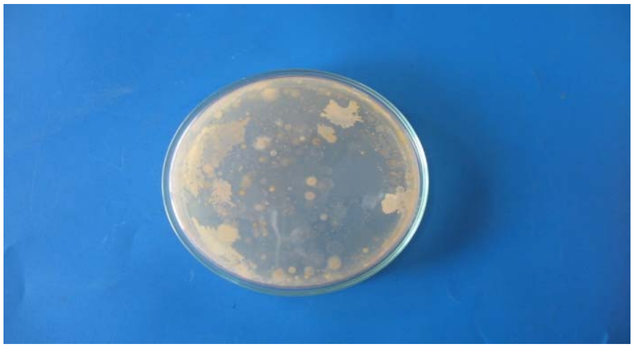

Fig 1: Bacterial diversity of retting pond

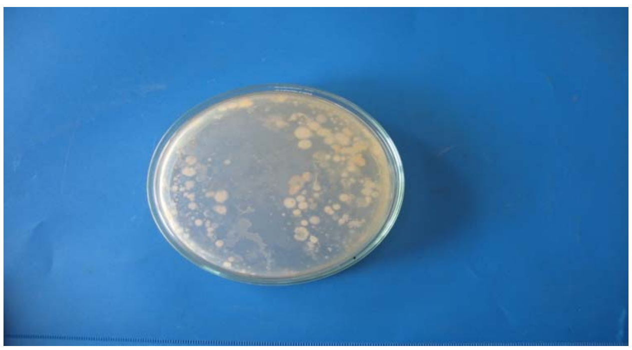

Fig 2: Bacterial diversity of estuary

Fig 3: Plate showing the presence of cellulolytic bacteria

5. References

1. Vinotha T, Maheswari UN. Optimization of cellulolytic bacteria from cellulose waste materials and its activity. Int. J. Pharm. Sci. Rev. Res, 2014; 26(2): 333-337.
2. Sexana S, Bahadur J, Varma A. Cellulose and hemi-cellulose degrading bacteria from termite gut andmound soils of India. Int. J. Microbiol, 1993; 33 (1): 55-60.
3. Nakamura K, Kappamura K. Isolation and identification of crystalline cellulose hydrolyzing bacterium and its enzymatic properties. J Ferment Technol, 1982, 60 (4):343-8.
4. Lee SM,.Koo YM. Pilot-scale production of cellulose using Trichoderma reesei Rut C-30 in fed-batch mode. J. Microbiol. Biotechnol, 2001; 11: 229-233.
5. Stewart B, Leatherwood JM. Derepressed synthesis of cellulase by *Cellulomonas*. J. Bacteriol, 1976; 128: 609-615.
6. Haggett KD, Choi WY, Dunn NW. Mutants of Cellulomons, which produce increased levels of β”glucosidase. Europ. J. Appl. Microbiol. Biotechnol, 1978; 6: 189 -191.
7. Gokhale DV, Soo-Han E, SrinivasanVR, Deobagkar DN. Transfer of DNA coding for cellulases from *Cellulomonas sp*. to *Bacillus subtilis* by protoplast fusion. Biotechnol. Lett, 1984; 6: 627-632.
8. Prasetsan P, Doelle HW. Nutrient optimization for cellulase biosynthesis by a newly isolated *Cellulomonas* sp. Mircen. J, 1987; 3: 33-44.
9. Kansoh AL, Essam SA, Zeinat AN. Biodegradation and utilization of bagasse with *Trichoderma reesei*. Polym. Degrad. Stab, 1999; 62: 273-278.
10. Ojumu T, SolomonV, Bamidele O, Betiku E, Layokun SK, Amigun B. Cellulase Production by *Aspergillus flavus* Linn isolate NSPR 101 fermented in sawdust, bagasse and corncob**.** African J. Biotechnol, 2003; **2** (6): 150–152.
11. Solomon BO, Amigun B, Betiku E, Ojumu T, Layokun SK. Optimization of Cellulase Production by *Aspergillus flavus* Linn Isolate NSPR 101 Grown on Bagasse. JNSChE, 1999; **16**: 61-68.
12. Immanuel G, Dhanusa R, Prema P, Palavesam A. Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. Int. J. Environ. Sci. Tech, 2006; 3(1): 25-34.
13. Gautam SP, Bundela PS, Pandey AK, Awasthi MK, Sarsaiya S. 2010. Screening of cellulolytic fungi for management of municipal solid waste. J App Sci Environ Sanit, (4): 391-395.
14. Devi AM, Pillai GN. Effect of pollution due to coconut husk retting on the species diversity of benthic communities of Cochin backwaters. Indian J. Fish, 1990; 37 (2): 145 -149

15. APHA (American Public Health Association), 1998. Standard methods for the examination of water and wastewater, WEF and AWWA, 20th Edition, USA.
16. Irfan M, Safdar A, Nadeem M. Cellulolytic bacteria from soil and optimization of celulase production and its activity, Turk J Biochem, 2010; 37: 287- 293.
17. Jayasankar NP. An appraisal on the biological extraction of coir. Pro Workshop Coir Res, Cochin, Kerala, 1985.
18. Perez J, Munoz-Dorado J, de la Rubia T, Martinez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Int Microbiol, 2002; 5 (2): 53–63.
19. Niranjane AJ, Madhou P, Stevenson TW. The effect of carbohydratecarbon sources on the production of cellulase by *Phlebia gigantean*. Enzyme Microb Technol, 2007; 40:1464-1468.
20. Hatami S, Alikhani HA, Besharati H, Salehrastin N, Afrousheh M, Yazdani ZJ. 2008. Investigation on Aerobic Cellulolytic Bacteria in Some of North Forest and Farming Soils. American-Eurasian J Agric and Environ Sci, 3(5):713-716.
21. Das A, Bhattacharya S, Murali L. Production of cellulose from a thermophilic Bacillus sp. isolated from cow dung. American- Eurasian J Agric Environ Sci, 2010; 8(6):685-691.
22. Otajevwo FD, Aluyi HS. Cultural conditons necessary for optimal cellulase yield by cellulolytic bacterial organisms as they relate to residual sugars released in broth medium. modern applied science, 2011; 5(3) :141-15
23. Rasi MRP, Mahalingam. PU. Screening and partial characterization of cellulose degrading bacteria from decayed sawdust. international journal of science and research, 2014; 3(8):328-331
24. Nandan BS. Current status and biodiversity modification in the coastal wetland ecosystems of India with objectives for its sustainable management. Proceedings of the Conserv-vision conference, University of Waikato, Hamilton, New Zealand, 2008.
25. Sharan RK. 1989. Physico-chemical condition of Ganga water in western Bihar. IBID, 20: 32-33.
26. Trivedy RK, Goel PK. Chemical and biological methods for water pollution studies, Enviromedia publication, Karad, Maharastra (India), 1994.
27. Devi D, Anandhi DU. Assessment of water quality for aquaculture- A case study of Madavara lake in Bangalore. Nat. Env.and Poll. Tech, 2009; 8(4): 755-760.
28. Ganapathi, Ecology of tropical waters. In***:***Proceedings of the symposium on algology. Edited by P. Kachroo. I.C.A.R., New Delhi, 1960.
29. Paliwal KV. Irrigation with saline water.,Water Technology Centre, Indian Agricultural Research Institute, New Delhi, 1972, IARI Monograph No.2.
30. Jakhrani AQ, Samo SR, Nizamani I. Impact of wastewater effluents on physico-chemical properties of groundwater. Sindh University Research Journal (Sci. Ser.), 2009; 41(1): 75–82.
31. Amdur MO, Doull J, Klaassen CD. The basic science of poisons. Toxicology, Pergamon Press. 1991, 1033.
32. Ronghu G. Study on the effect of effluent pollution by dying and staining factories on the pregnancy outcomes of residents. J.Environ.Health, 1994; 11(2):87-88
33. Taylor EW. The examination of water and water supplies, Churchill, Blankiston, 1958.
34. Wagner RH. Environment and Man, Norton and Company, New York, 1974, 28.
35. 35. Lindsy T, Neese S, Thomas D. Pollution Prevention. Water Qual. Inter, 1960; 4: 32-36.
36. Speer E. Wastewater in Milk and dairy product technology. Marcel Dekker, INC, New York, U.S.A, 1995,447.
37. Raj A, Reddy MM, Krishna, Chandra, R. Decolourisation and treatment of pulp and paper mill effluent by lignin-degrading *Bacillus sp.* J. Chem. Technol. Biotechnol, 2007; 82: 399-406
38. Helfrich LA, Weighmann DL, Hipkins P, Stinson ER. Pesticides and aquatic animals:A guide to reducing impacts on aquatic system. Fisheries and wild life sciences. Pub.Virginia University Blacksbury Virginia, 1996, 613.
39. Battaglin W, Fairchild J. Potential toxicity of pesticides measured in Mid western streams to aquatic organisms. Water Science and Technology, 2002; 45(9): 95-103