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Biodegradation of cellulosic and lignocellulosic waste by *Pseudoxanthomonas* sp R-28

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ABSTRACT

A microbial consortium, designated Con R, was established by successive sub-cultivation which can degrade 83% of filter paper after 15 days of incubation over control. Among the 14 bacterial isolates obtained from Con R, only bacterial isolate (R-28) was capable of degrading filter paper. Based on 16S rRNA gene sequence, R-28 was identified as *Pseudoxanthomonas* sp R-28. After 5 days of incubation, degradation efficiencies of *Pseudoxanthomonas* sp R-28 on filter paper and pure cellulosic waste were 96% and 95% respectively as compared to control. *Pseudoxanthomonas* sp R-28 also degraded 60% of non-pretreated rice straw after 7 days as compared to control. The degradation kinetics through a modified logistic model showed high correlation coefficient (R^2) of 0.965 and 0.665 for cellulosic and rice straw waste degradation respectively. Micro scale structural analysis showed the development of fissures and gaps over time which further supported the degradation potential of *Pseudoxanthomonas* sp R-28.

1. Introduction

A key step in the global carbon cycle is the hydrolysis of the cellulose, which is the most abundant renewable natural organic matter on earth (Wilson, 2011). Annually, many billion tons of organic wastes are produced worldwide as residues from agricultural activities, industrial food processing and municipal solid waste (Bayer, Lamed, & Himmel, 2007). Cellulose is the major component of this waste accounting for 40–50% on dry weight basis. The half-life of cellulose at neutral pH is estimated to be several million years.

At present this waste is primarily disposed off either by land filling or by composting. Though degradative environment of composting is well studied and efficient (Hansgate, Schloss, Hay, & Walker, 2005), the uncontrollable landfill environment is often less efficient (Bayer et al., 2007). Contamination of groundwater and landfills rapidly filling to its capacity are other major concerns. Moreover, mostly in Asian countries, burning of the waste in open fields causes the release of green house gases thus polluting the environment (Murali, Shrivastava, & Saxena, 2010). Consequently, the use of microorganisms in order to remove, reduce or ameliorate these potential polluting materials is a real environmental challenge, which could be solved by a focused research concerning efficient methods applied in biological degradation processes (Annamalai, Rajeswari, Elayaraja, & Balasubramanian, 2013; Bayer et al., 2007; Petre, Zarnea, Adrian, & Gheorghiu, 1999).

Unique stable structure of cellulose makes it recalcitrant to microbial attack (Huang, Chen, Lin, Hsu, & Chen, 2010; Lei, Li, & Gu, 2012). Thus poor degradation efficiency is one of the major bottlenecks associated with cellulosic waste biodegradation. The anaerobic degradation of cellulosic wastes has been intensively studied particularly during composting and in rumen (Russell, Muck, & Weimer, 2009; Schloss, Hay, Wilson, Gossett, & Walker, 2005). Aerobic degradation of cellulosic waste could be an attractive strategy which is poorly understood so far. Only few aerobic bacteria have been reported which can degrade lignocellulose poorly that underscores the need to characterize the more efficient aerobic system. Kinetic modeling offers a powerful tool to model the substrate utilization. Logisitc equation has been used to study the growth kinetics or degradation studies of 2,4-D or anaerobic utilization of cellulose (Langner et al., 1998; Yu, Chen, Tong, Li, & Li, 2012). Therefore, this study aims to characterize an aerobic microbial system which could have the potential to degrade organic solid waste (rice straw). The performance of the biodegradation process has been evaluated by taking into account both the reaction kinetics and morphological/structural changes.

2. Materials and methods

Whatman filter paper No. 1 was purchased from Schleicher & Schull, Whatman International Ltd., Maidstone, UK. Molecular

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biology reagents were purchased from Thermo Scientific, USA. All other chemicals and reagents were of analytical grade and purchased from HiMedia, India. Rice straw was procured from the agricultural field, Punjab.

2.1. Development of aerobic consortium

Soil samples were collected from semi-desert soil of northern India as described previously (Kumar & Khanna, 2014) and these soil samples were used to develop aerobic consortia by repeated enrichment technique at 37 °C in Bushnell Haas broth (BHB) (Kumar, Joshi, Kashyap, & Khanna, 2011) containing 0.5% (w/v) filter paper as the only added carbon source. The developed consortia were screened for degradation of filter paper by incubating the inoculated flasks on a rotary shaker (150 rpm) at 37 °C and measuring cellular growth and analyzing filter paper (cellulose) degradation. At each time point, samples were withdrawn in duplicate to measure cellular growth and to analyze cellulose utilization. Control flask included BHB + soil + filter paper, which was autoclaved to determine the abiotic loss of cellulose. The process was repeated for another six week at an interval of 15 days each to acclimatize the consortium. Developed consortium (Con R) was inoculated at 6% (v/v) in BHB + FP (0.5%, w/v) and incubated at 37 °C. After regular interval of time, growth and filter paper degradation was determined.

2.2. Isolation and screening of bacterial strains for cellulose degradation

Bacterial strains were isolated from the developed aerobic consortium by dilution technique on basal agar plates containing carboxy methyl cellulose (CMC; 0.5% w/v) as sole source of carbon. Bacterial isolates were then picked, purified and grown in 2 ml BHB medium supplemented with CMC (0.5%; w/v). Selected bacteria were also inoculated into BHB + filter paper (0.5%; w/v) to determine their ability to degrade filter paper.

2.3. Estimation of growth and filter paper degradation

After regular interval of time, total protein was determined as an indicator of growth as described by Itzhaki and Gill (1964). Residual filter paper in each flask (both experimental and control) was centrifuged (15 000 × *g*, 30 min at 4 °C) and acidified by addition of concentrated H_2SO_4 at each time point until the final concentration of H_2SO_4 reached 70% (v/v) (Velichkov (1992). The acidified flasks were kept at 30 °C for 1 h to complete the acid digestion of filter paper and an aliquot (50 μl) of the sample (in duplicate) was withdrawn and mixed with 3 ml of anthrone reagent (0.2%, w/v). The tubes were then incubated in a boiling water bath for 10 min and immediately cooled to room temperature in an ice bath. Absorbance was measured at 630 nm by spectrophotometer (Hitachi, Japan). Experiment was repeated three times.

2.4. Molecular identification of cellulose degrading bacteria

Chromosomal DNA of the selected bacteria was extracted as described by Kumar and Khanna (2010). 16S rRNA gene was amplified using genomic DNA as template with universal primers E8F (AGAGTTTGATCCTGGCTCAG) and reverse primer E1492R (GGTTACCTTGTTACGACTT) as described in literature (Bond, Hugenholtz, Keller, & Blackall, 1995). The PCR reactions were prepared with 10X buffer, 0.2 mM deoxynucleoside triphosphate (dNTP), 1 U Taq polymerase (all from Life Technologies, India), and 0.5 μM for each primer (Sigma, India) to yield final volumes of 25 μl, using 35 cycles of 94 °C (60 s), 55 °C (60 s), and 72 °C (60 s) with an initial denaturation at 94 °C (3 min) and a final extension at 72 °C (8 min). Sequencing was done by Amnion Biosciences, Bangalore, India and then BLAST searched through the NCBI GenBank database. Phylogenetic tree was constructed using molecular evolutionary genetics analysis (MEGA) software with 1000 bootstrap replicates (Tamura, Stecher, Peterson, Filpski, & Kumar, 2013).

2.5. Scanning electron microscope (SEM) analysis

SEM (Supra 55; Zeiss, Germany) was used to study the changes in the morphological characteristics of filter paper at different time intervals (Institute of Minerals and Materials Technology, Bhubaneswar). A thin layer of gold was coated to make the samples conductive. The accelerating voltage of the microscope was kept in the range 20–30 kV.

2.6. Biodegradation of cellulose waste and agro residue

Paper sheets (BILT Copy Power, BILT India) was used as cellulosic waste paper. Characteristics of waste paper, as defined by manufacturer were: 80GSM, brightness (93% Elerpho) and stiffness (2.6 machine direction, 1.6 cross machine direction). Agro residue i.e. rice straw and waste paper were cut manually to achieve particle size of 0.2–0.3 cm in length. Rice straw was further grinded in stone grinder and sieved to collect particle size of 0.35–0.5 mm and collected particles were washed 2–3 times in tap water to remove the soil particles. Sterilized BHB medium supplement with either waste paper or rice straw as sole carbon source was inoculated with selected bacteria. Control flask was inoculated with killed bacteria. Flasks were incubated at 37 °C under shaking at 150 rpm. After regular interval of time, whole flask was used to determine the growth and utilization of cellulosic waste. Residual rice straw was estimated as described by (Du et al., 2015). The degradation ratio of the rice straw was defined as the ratio of the weight of degraded rice straw compared to the weight of total rice straw added at time zero and calculated by the following formula:

$$= \{1 - m(\text{Residual Rice Straw})/m(\text{Total Rice Straw})\} \times 100 \quad (1)$$

where m is mass.

2.7. Kinetic modeling

A modified logistic equation (Zwietering, Jongenburger, Rombouts, & van't Riet, 1990) was adopted to describe the degradation kinetics:

$$S = S_0 \left\{1 - 1/\{1 + \exp[4R_m/S_0(\lambda - t) + 2]\}\right\} \quad (2)$$

where S is substrate concentration at time t (g/L), t is incubation time (h), S_0 is initial substrate concentration (g/L), λ is lag time (d), R_m is maximum substrate degradation rate (g/L/h).

The rate of substrate degradation r_s (g/L/h) was described as:

$$r_s = -4R_m \exp[4R_m/S_0(\lambda - t) + 2]/\{1 + \exp[4R_m/S_0(\lambda - t) + 2]\}^2 \quad (3)$$

2.8. Nucleotide sequence submission

The nucleotide sequence of 16S rRNA of the bacterial strain reported in this study was deposited in the GenBank database with the following accession number KP876051.

3. Results and discussion

3.1. Development of an aerobic consortium

Aerobic bacterial consortium (Con R) developed by enrichment technique was capable of growth on mineral salt medium