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Biochemical characterization of thermophilic lignocellulose degrading enzymes and their potential for biomass bioprocessing

Vasudeo Zambare[1], Archana Zambare[1], Kasiviswanath Muthukumarappan[2], Lew P. Christopher[1]

[1] Center for Bioprocessing Research & Development, South Dakota School of Mines and Technology, Rapid City 57701, SD, USA.
[2] Center for Bioprocessing Research & Development, South Dakota State University, Brookings 57007, SD, USA.

Abstract
A thermophilic microbial consortium (TMC) producing hydrolytic (cellulolytic and xylanolytic) enzymes was isolated from yard waste compost following enrichment with carboxymethyl cellulose and birchwood xylan. When grown on 5% lignocellulosic substrates (corn stover and prairie cord grass) at 60^0C, the thermophilic consortium produced more xylanase (up to 489 U/l on corn stover) than cellulase activity (up to 367 U/l on prairie cord grass). Except for the carboxymethyl cellulose-enriched consortium, thermo-mechanical extrusion pretreatment of these substrates had a positive effect on both activities with up to 13% and 21% increase in the xylanase and cellulase production, respectively. The optimum temperatures of the crude cellulase and xylanase were 60^0C and 70^0C with half-lives of 15 h and 18 h, respectively, suggesting higher thermostability for the TMC xylanase. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the crude enzyme exhibited protein bands of 25-77 kDa with multiple enzyme activities containing 3 cellulases and 3 xylanases. The substrate specificity declined in the following descending order: avicel>birchwood xylan>microcrystalline cellulose>filter paper>pine wood saw dust>carboxymethyl cellulose. The crude enzyme was 77% more active on insoluble than soluble cellulose. The K_m and V_{max} values were 36.49 mg/ml and 2.98 U/mg protein on avicel (cellulase), and 22.25 mg/ml and 2.09 U/mg protein, on birchwood xylan (xylanase). A total of 50 TMC isolates were screened for cellulase and xylanase secretion on agar plates. All single isolates showed significantly lower enzyme activities when compared to the thermophilic consortia. This is indicative of the strong synergistic interactions that exist within the thermophilic microbial consortium and enhance its hydrolytic capabilities. It was further demonstrated that the thermostable enzyme-generated lignocellulosic hydrolyzates can be fermented to bioethanol by a recombinant strain of *Escherichia coli*. This could have important implications in the enzymatic breakdown of lignocellulosic biomass for the establishment of a robust and cost-efficient process for production of cellulosic ethanol. To the best of our knowledge, this work represents the first report in literature on biochemical characterization of lignocellulose-degrading enzymes from a thermophilic microbial consortium.

Keywords: Cellulase, Xylanase, Thermophilic microbial consortium, Bioethanol.

1. Introduction

Although significant progress has been recently made towards commercialization of cellulosic ethanol, there are still technological challenges that need to be addressed. It is now recognized that cellulose is the rate-limiting substrate in bioethanol production and new, more efficient enzymes are required to overcome the cellulose recalcitrance to biodegradation. Improving current efficiency and understanding of cellulosic bioethanol requires a variety of new capabilities including cultivating thermophilic microbial consortia which can produce robust enzyme systems with high hydrolytic potential for cellulose degradation [1]. Hence, the search for and discovery of novel thermostable enzymes with enhanced capabilities for cellulose degradation may lead to significant improvements in the bioethanol process [2]. The tolerance of high temperatures improves the enzyme robustness and increases the enzyme reaction rates needed for industrial-scale processes thereby decreasing the amount of enzyme needed [3]. Added benefits are reduced likelihood of culture contamination, improved substrate accessibility to cellulases and reduced viscosity of feedstock allowing the use of higher solids loadings [4]. From this perspective, the enrichment from nature of thermophilic microbial communities with high cellulolytic activity is useful in the identification of novel enzymes with functions that enhance our fundamental understanding of microbial cellulose degradation and help eliminate the current inefficiencies in the bioethanol production process. The extreme environmental resistance of thermophilic microbial consortia permits screening, isolation and exploitation of novel cellulases and xylanases to help overcome these challenges. Reports are available in literature on the use of thermophilic cultures for production of ethanol from lignocellulosics [5-7]. Anaerobic digestion of lignocellulosic waste using thermophilic microorganisms for composting, waste disposal and biogas production has been widely reported [8-11]. Furthermore, the interest in thermophiles has increased due to their potential use in the production of value-added bioactive compounds such as enzymes and antibiotics [12, 13]. Thermophilic cellulase-producing microorganisms have been isolated from a variety of natural habitats including hot springs [14, 15] and composting heaps [16, 17]. However, cellulase production has been mainly described for single thermophilic microorganisms such as Clostridium sp [18, 19], Thermoascus aurentiacus [20], Sporotrichum thermophile [21], Paenibacillus sp. [22], Brevibacillus sp. [23], Anoxybacillus sp. [24], etc. Recently, strains of cellulolytic thermophiles, Bacillus and Geobasillus, have been also isolated and characterized in our laboratories [25, 26]. Nevertheless, only a few reports are available on the use of thermophilic consortia for cellulase and xylanase production [7, 27]. These reports, however, lack information on the biochemical and kinetic properties of the secreted enzymes. Such information may be useful in gaining better understanding of the lignocellulose biodegradation in relation to the enzyme system produced by the microbial community. The focus of this work was on the characterization of cellulose- and xylan-degrading enzymes from a thermophilic microbial consortium obtained by enrichment of yard waste compost as a source.

2. Materials and methods

2.1 Chemicals and reagents

All chemicals and media used in this study such as Nutrient broth, microcrystalline cellulose (MCC), carboxymethyl cellulose (CMC), birchwood xylan (BWX), 3,5-dinitrosalicylic acid (DNSA), avicel, sodium dodecyl sulphate, Bradford reagent and protein molecular weight markers were procured from Sigma (St. Louis, MO, USA). Whatman filter paper No. 1 and silver staining kit (SilverSNAP) were purchased from Fisher Scientific (Pittsburgh, PA, USA) and Thermo Fisher Scientific (Rockford, IL, USA), respectively.

2.2 Lignocellulosic substrates

Pine wood saw dust (PWSD) was obtained from a local saw mill in Rapid City, SD. Corn stover (CS) and prairie cord grass (PCG) were thermo-mechanically pretreated using a single screw extruder (Brabender Plasti-corder Extruder Model PL2000, Hackensack, NJ). During extrusion, a screw speed of the extruder of 100 rpm and a barrel temperature of 100°C was maintained [28].

2.3 Sample collection

Samples of yard waste compost (YWC) and finished yard waste compost (FYWC) were collected from the Rapid City Land Filling and Recycling Center (Rapid City, SD, USA). The YWC I and II samples were taken from the composting heap bottom and top, respectively, while FYWC was sampled from the processed compost. The compost temperatures were measured during sampling with a deep fryer

thermometer. Samples were collected in sterile bottles by digging 1.5 ft x 1.5 ft area of the compost and bottles were stored at 4^0C.

2.4 Enrichment of thermophilic microbial consortia

All three compost samples (1% w/v) were inoculated into 500-ml Erlenmeyer flasks containing 100 ml sterile Nutrient broth supplemented with 0.2% (w/v) MCC or 0.2% (w/v) BWX and incubated at 60^0C under shaking (155 rpm) for 8 days. During enrichment, samples were removed aseptically at regular intervals of 24 h for up to 8 days and analyzed for pH, cell density, cellulase and xylanase activities, reducing sugars (RS) and protein content by methods described below. The enriched thermophilic microbial consortia (TMC) were preserved as glycerol stocks at -80^0C.

2.5 Isolation of single cultures from thermophilic microbial consortia

Individual cultures from the MCC- and BXW-enriched consortium were isolated by the serial dilution method [29]. All isolated pure cultures were spot inoculated on MCC and BWX nutrient agar plates and incubated at 60^0C for 72 h. After incubation, all plates were flooded with 0.1% Congo red followed by destaining with 1M NaCl [30]. Positive cultures showed a zone of clearance around the cell growth. Cultures with a measurable clear zone were inoculated in a production medium as given in section 2.8 with PCS as carbon source. All flasks were incubated at 60^0C and 150 rpm for 120 h and the enzyme activities determined thereafter.

2.6 Enzyme assays

The cellulase and xylanase activity were determined by the assay method of Dutta et al. [31] and Cheng et al. [32], respectively. The supernatant containing the enzyme (0.5 ml) was incubated with 0.5 ml 1% (w/v) CMC (cellulase) or 0.5 ml 1% (w/v) BWX (xylanase) in phosphate buffer (100 mM, pH 7.0) at 60^0C for 30 min. The RS were measured with DNSA reagent [33] using glucose (cellulase) or xylose (xylanase) as standard. One unit (U) of enzyme activity was expressed as the amount of enzyme liberating 1 µM of glucose (cellulase) or xylose (xylanase) equivalents per min under the assay conditions.

2.7 Morphology of microbial consortia

The morphology of growing BWX-enriched TMC was observed on cellulose, xylan, pretreated CS (PCS) and pretreated PCG (PPCG) from 2.6 mm working distance using a scanning electron microscope (SEM) model SUPRA40VP (Zeiss, Thornwood, NY, USA) equipped with a SE2 detector. Samples were prepared according to DeXaun et al. [34].

2.8 Enzyme production by thermophilic microbial consortia

Lignocellulosic substrates (CS, PCS, PCG and PPCG) were used as carbon source at 0.5% (w/v) in 500-ml Erlenmeyer flasks containing 100 ml of medium (pH 7.0) that was composed of (w/v): 0.02% yeast extract, 0.05% K_2HPO_4, 0.025% KH_2PO_4, 0.01% $CaCl_2$. Independent inoculations were carried out with MCC- and BWX-enriched TMC isolated from YWC-II and incubated at 155 rpm and 60^0C for 120 h. During incubation, samples from the lignocellulosic hydrolyzates were removed aseptically at regular intervals of 48 h for up to 120 h and analyzed for pH, protein content, cellulase and xylanase activity.

2.9 Enzyme characterization

The crude enzymes of TMC were characterized with respect to their activity under different pH (3-10) and temperature (30-100^0C) conditions. The enzyme thermostability was determined at 50-80^0C for up to 3 h. The substrate specificity of the crude enzymes was examined against 10 mg/ml MCC, avicel, CMC, Whatman filter paper No. 1 (filer paper), BWX and PWSD. The enzyme kinetic studies for cellulase and xylanase (K_m and V_{max}) were performed with 1-10 mg/ml of CMC and BWX, respectively [35]. The crude cellulase and xylanase were subjected to denaturation using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% (w/v) gels by the method of Holt and Hartman [36]. After electrophoresis, gels were silver-stained for protein [37] using protein molecular weight markers of 10-225 kDA. Gel electrophoresis on 1% (w/v) CMC and 1% (w/v) BWX was run and analyzed by zymogram analysis [36]. Gels were stained for cellulase activity in 0.1% (w/v) Congo Red solution at room temperature for 30 min. The activity band was observed as a clear colorless area, depleted of CMC, against a red background when destained in 1M NaCl solution.

2.10 Ethanol fermentation

Lignocellulosic hydrolyzates obtained following incubation of TMC on PCS and PPCG for 120 h served as feedstock for ethanol production with a recombinant pentose and hexose fermenting *Escherichia coli* KO11. This strain was a kind gift from Dr. Lonnie Ingram, University of Florida, Gainesville, FL, USA. Seed cultures of *E. coli* were developed in 250-ml flasks containing 100 ml Luria broth with 10% (w/v) glucose incubated at 30°C and 150 rpm for 24 h. For fermentation, 1 ml inoculum of *E. coli* KO11 was added to 100 ml serum bottles containing 25 ml lignocellulosic hydrolyzate (pH 6.0). The serum bottles were incubated at 30°C and 150 rpm for 120 h. During fermentation, samples were removed aseptically at regular intervals of 48 h for up to 120 h and analyzed for pH, glucose, xylose and ethanol.

2.11 Analyses

Glucose, xylose and ethanol were measured with 2700 Biochemistry Analyzer (YSI Life Sciences, Yellow Spring, Ohio, USA) as per the manufacturer's instructions. Protein was estimated by the Bradford method using bovine serum albumin as standard [38]. All experiments were run in duplicate and standard deviations (SD) were calculated using Microsoft Excel and results were presented as average ± SD.

3. Results and discussion

3.1 Compost characterization

A summary of the YWC characteristics is shown in Table 1. All compost samples were of black color owing to the formation of humic substances, carbon dioxide and volatile organic acids [39, 40]. The FYWC sample had a lower temperature because of the heat released during its processing. The pH was above 8 in the YWC-I and –II samples, however, the FYWC sample showed acidic pH due to the formation of organic acid and reduced levels of ammonia after compost processing [41, 42].

Table 1. Characterization of yard waste compost samples

Specifications	YWC[a]-I	YWC-II	FYWC[b]
Location	Bottom of heap	Top of heap	Centre of heap
Texture	Coarse	Coarse	Fine
Color	Black	Black	Black
Temperature	63^0C	79^0C	37^0C
pH	8.2	8.6	6.6
Moisture (%)	20.6%	20.5%	23.5%

[a]YWC, yard waste compost; [b]FYWC, finished yard waste compost

3.2 Compost enrichment

Based on analyses of the compost samples in the MCC and BWX enrichment medium, the YWC-II sample was found to be the best potential source of TMC producing the highest cellulase (238 U/l) and xylanase (471 U/l) activity after 48 h of incubation (data not shown). Cellulolytic and xylanolytic bacteria have been frequently sourced from compost ecosystems [43, 44] as both cellulose and xylan are present in the form of waste paper and plant residues as major constituents of municipal and yard waste [45]. For instance, a strain of *Scytalidium thermophilum* producing cellulolytic and hemicellulolytic enzymes was isolated by enrichment of composting soil [46]. Ryckeboer et al. [47] isolated thermophilic microflora from biowaste in a CMC-supplemented medium. Chang et al. [48] isolated a cellulolytic thermophilic *Bacillus* sp. from brassica waste compost whereas Guisado et al. reported xylanase activity (2.45 U/ml) during enrichment of composting piles [49].

3.3 Morphology of microbial consortia

The SEM images revealed (Figure 1) that the substrate surface was populated with TMC growing cells which caused a progressive depolymerization and solubilization of lignocellulosic biomass. Likewise, the adsorption of *Paenibacillus curdlanolyticus* B-6 cells to xylan was analyzed by SEM indicating that with time the lignocellulosic substrates rendered more susceptible to reaction with the microbial hydrolytic enzymes [50].

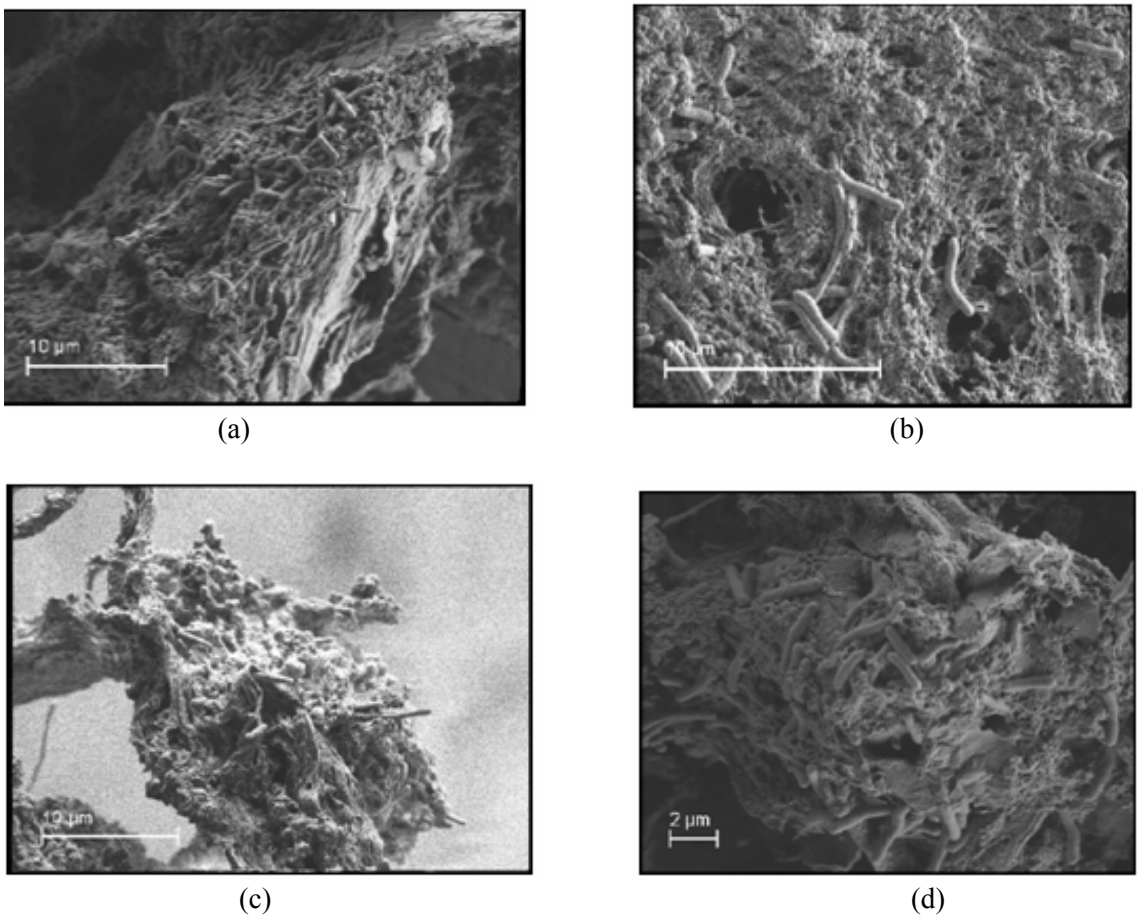

Figure 1. SEM images indicating adhesion of cells from the birchwood-enriched thermophilic microbial consortium to cellulose (a), xylan (b), pretreated corn stover (c), pretreated prairie cord grass (d)

Table 2. Cellulase and xylanase production by the thermophilic microbial consortium grown on lignocellulosic substrates

Consortium and substrates	Enzyme activities (U/l)	
	Cellulase	Xylanase
MCC-enriched consortium		
Corn stover	312 ± 10.5	489 ± 6.4
Pretreated corn stover	201 ± 6.5	552 ± 0.9
Prairie cord grass	367 ± 6.5	360 ± 9.4
Pretreated prairie cord grass	344 ± 13.0	400 ± 5.0
BWX-enriched consortium		
Corn stover	219 ± 6.5	452 ± 17.2
Pretreated corn stover	265 ± 6.5	485 ± 5.2
Prairie cord grass	293 ± 16.4	308 ± 26.1
Pretreated prairie cord grass	307 ± 2.1	308 ± 10.8

Activities are average of duplicate determinations ± SD. Growth conditions: 60^0C, 0.5% (w/v) substrate, pH 7, 120 h; MCC, microcrystalline cellulose; BWX, birchwood xylan

3.4 Enzyme production by thermophilic microbial consortia

When grown on 5% lignocellulosic substrates (corn stover and prairie cord grass) at 60^0C, the TMC produced more xylanase than cellulase activity (Table 2). The lower cellulase activity might be due to the highly crystalline nature of MCC used in the enrichment studies as well as the microbial diversity in TMC. Production of higher xylanase activity (189.7 U/ml) than cellulase activity (2.79 U/ml) using a fungal consortium on wheat bran was reported by Ikram-ul-Haq et al. [51]. Corn stover induced more xylanase activity of up to 489 U/l (Table 2) whereas prairie cord grass was the better substrate to produce higher cellulase activity (367 U/l). Except for the cellulose-enriched consortium, thermo-mechanical pretreatment of these substrates had a positive effect on both activities with up to 13% and 21% increase of the xylanase and cellulase production, respectively.

3.5 Enzyme characterization

The TMC crude enzyme was active in a broad pH range (pH 3-10), however, maximum cellulase and xylanase activities were obtained at pH 4 (Figure 2) Interestingly, a peak in the cellulase activity was also observed at pH 7 and pH 10. This could be due to the fact that the TMC contained diverse microbes that secrete multiple enzymes with different pH optima. As no studies in literature appear to be available on the characterization of enzymes from TMC, the results in this work could not be discussed in the context of a relevant comparison to other reports. However, literature survey for single thermophilic cultures suggests that cellulases exhibit maximum activity at both acidic and alkaline pH [52, 53].

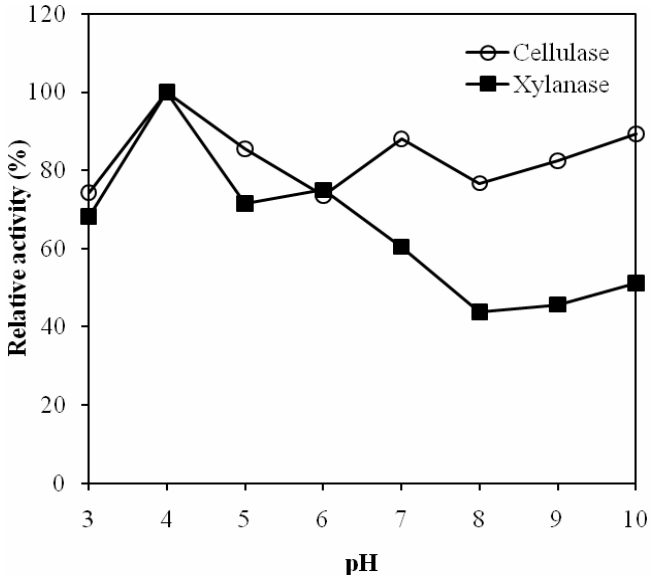

Figure 2. Effect of pH (at 60^0C) on the cellulase and xylanase activity of the carboxymethyl cellulose-enriched and birchwood xylan-enriched thermophilic microbial consortia

The crude enzyme from TMC was active in a broad temperature range (from 40 to 80ºC) with a temperature optimum of 60^0C, for cellulase, and 70^0C, for xylanase (Figure 3). Between 50 and 70^0C, however, both enzymes retained more than 80% of their maximum activity. Bajaj et al. [54] reported 60^0C as temperature optimum for a cellulase from *Bacillus* sp. M-9 whereas xylanases from *Bacillus* sp. had their optimum activity at 60 to 80^0C [55-57].

The TMC enzymes retained 98% of cellulase activity after incubation at 50ºC for 1 h, and 77%, after incubation at 60^0C for 3 h (Figure 4a). On the other hand, the residual xylanase activity after 1 and 3 h of incubation was 99%, at 50^0C, and 89%, at 60^0C, respectively (Figure 4b). At 60^oC, the half-life of cellulase and xylanase was 15 h and 18 h, respectively, suggesting a higher thermostability of the TMC xylanase. Likewise, a *Bacillus* sp strain 3M xylanase retained 100% of activity for at least 3 days at 55^0C and retained 47% activity at 80^0C [52] whereas the residual activity of a *Caldibacillus cellulovorans* cellulase was 83% after incubation at 70°C for 3 h, with half-lives of 32 min at 80°C, and 2 min at 85°C [53].

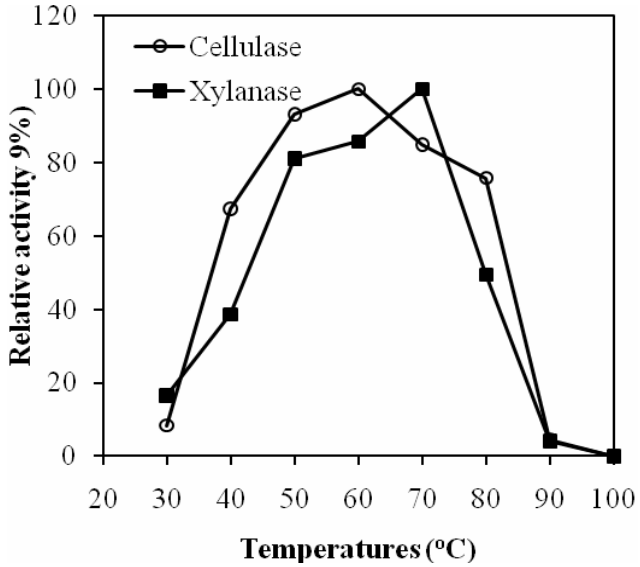

Figure 3. Effect of temperature (at pH 7) on the cellulase and xylanase activity of the carboxymethyl cellulose-enriched and birchwood xylan-enriched thermophilic microbial consortium

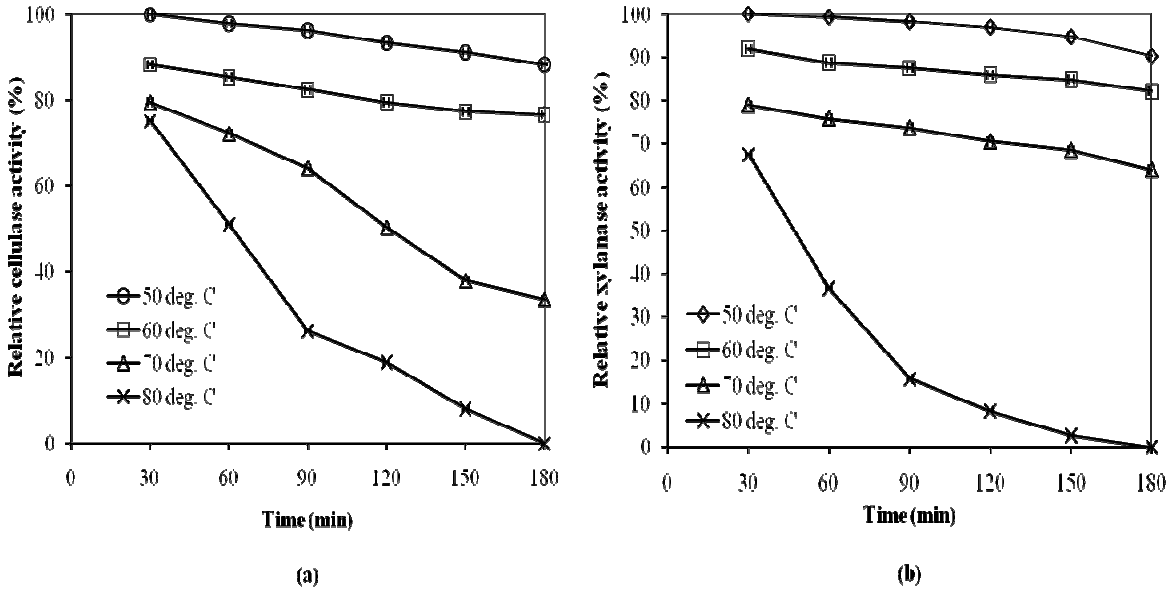

Figure 4. Thermostability of the carboxymethyl cellulose-enriched (a) and birchwood xylan-enriched (b) thermophilic microbial consortia

The crude enzyme of TMC migrated on SDS-PAGE as several bands with different molecular weights (Figure 5). Zymogram analysis revealed 3 bands staining for cellulase and xylanase activity each, where clear hydrolytic activity zones were formed against dark background. The cellulase proteins migrated with molecular masses of 60, 35 and 27 kDa whereas the molecular masses for the xylanase proteins were 75, 45 and 35 kDa (Figure 5). The molecular masses of the TMC enzymes reported here are in agreement with those available in literature for individual microorganisms: 27 kDa for a *Thermotoga maritima* cellulase [58]; 45 kDa for a *B. licheniformis* xylanase [59]; 60 kDa for a *Bacillus* sp. cellulase [60]; and 75 kDa for a recombinant *E. coli* xylanase [61]. In our study, only one mass protein of 35 kDa showed both cellulase and xylanase activity. A 35 kDa protein was reported for a *T. aurentiacus* cellulase [62], a *B. subtilis* B230 xylanase [63] and a *Postia placenta* multienzyme cellulase and xylanase complex [64].

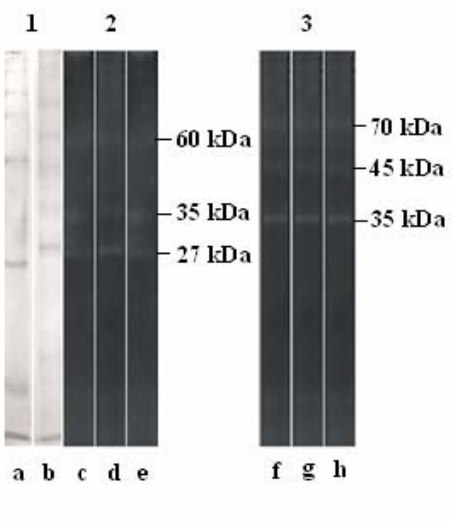

Figure 5. SDS-PAGE of the crude enzyme from the thermophilic microbial consortium
1, silver staining with broad range protein molecular weight markers (a), crude enzyme mixture (b); 2, zymogram of cellulase with carboxymethyl cellulose at pH 4 (c), pH 7 (d), pH 10 (e); 3, zymogram of xylanase with birchwood xylan at pH 4 (f), pH 7 (g), pH 10 (h)

The crude TMC enzyme exhibited the greatest substrate affinity for avicel followed by BWX, MCC, filter paper, PWSD and CMC (Table 3). It was 77% more active on insoluble cellulose (avicel) than soluble cellulose (CMC). On CMC, the TMC cellulase had K_m and V_{max} values of 36.49 mg/ml and 2.98 U/mg protein, respectively, whereas on BWX, K_m and V_{max} values of 22.25 mg/ml and 2.09 U/mg protein, respectively, were determined (data not shown). For single cultures, a cellulase of *Coptotermes formosanus* had K_m and V_{max} values of 1.90 mg/ml and 148.2 U/mg protein, respectively, on CMC [65]. Nakamura et al. [66] reported a K_m of 3.3 mg/ml CMC and a V_{max} of 1100 µmole/mg protein for a xylanase from *Bacillus* sp.

Table 3. Substrate specificity of the crude enzyme from the thermophilic microbial consortium

Substrates	Enzyme	Relative activity (%)
Avicel	Cellulase	100
Xylan	Xylanase	96
MCC	Cellulase	94
PWSD	Xylanase	33
Filter paper	Cellulase	28
CMC	Cellulase	23

Relative activity was expressed as percentage of maximum activity; MCC, microcrystalline cellulose; PWSD, pine wood saw dust; CMC, carboxymethyl cellulose

The lignocellulosic hydrolyzates of PCS and PPCG containing glucose (up to 1.34 g/l) and xylose (up to 0.24 g/l) were fermented to ethanol by a recombinant *E. coli* KO11 and similar ethanol yields were obtained (data not shown). Assimilation of both pentose and hexose sugars was found in both hydrolyzates, as also reported for *E. coli* KO11 on hydrolyzates from corn cobs, sugar cane bagasse and other agricultural residues [67-69].

3.6 Enzyme production by single isolates from the thermophilic microbial consortia

A total of 25 isolates from the MCC-enriched consortium and 25 isolates from the BWXenriched consortium were screened for extracellular cellulase and xylanase secretion (Figure 6).

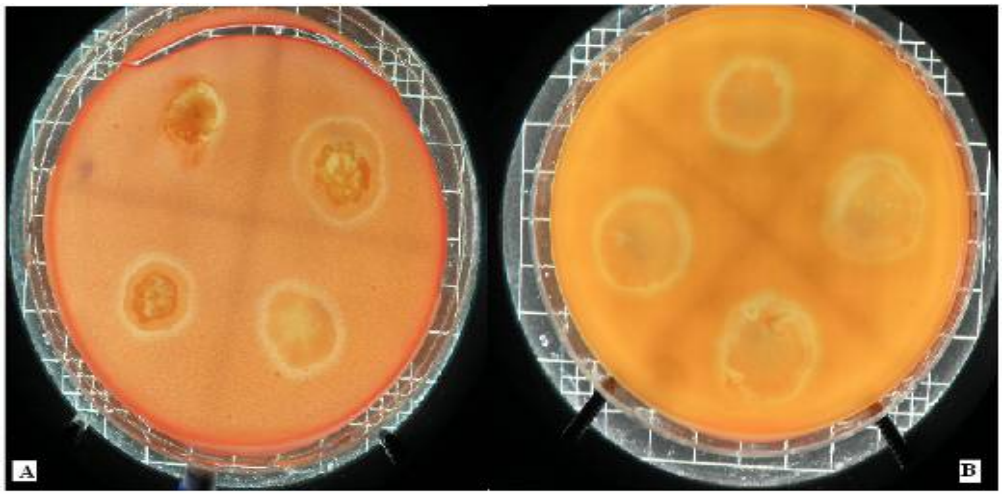

Figure 6. Isolation of cellulase (A, 1% MCC agar plates) and xylanase (B, 1% BMX agar plates) producing single cultures from the thermophilic microbial consortium (MCC, microcrystalline cellulose; BMX, birchwood xylan)

Based on the screening results, 10 isolates with measurable clear zones from the MCC-enriched microbial consortium, and 8 isolates from the BWX enriched consortium were selected for cellulase and xylanase production in liquid fermentation. All isolates showed lower enzyme activities (Table 4) when compared to the respective enrichment thermophilic consortia (Table 2). This clearly indicates that strong synergistic interactions exist within the microbial consortium which enhance its hydrolytic capabilities. Shivakumar and Nand [70] reported increased pectin degradation by a microbial consortium as compared to individual cultures.

Table 4. Cellulase and xylanase activities of individual cultures isolated from the thermophilic microbial consortium

Isolates	Enzyme activities (U/L)	
	Cellulase	Xylanase
Single isolates (MCC-enriched consortium)		
MCC-1	164 ± 6.5	223 ± 10.4
MCC-2	187 ± 0	53 ± 0
MCC-3	141 ± 13	271 ± 5.2
MCC-4	117 ± 6.5	104 ± 20.9
MCC-7	11 ± 13	134 ± 10.4
MCC-10	182 ± 6.5	82 ± 10.4
MCC-22	191 ± 6.5	75 ± 20.9
MCC-23	150 ± 13	159 ± 15.6
MCC-24	94 ± 26.1	86 ± 5.2
MCC-25	141 ± 39.2	164 ± 20.9
Single isolates (BMX-enriched consortium)		
BWX-1	191 ± 19.6	101 ± 26.1
BWX-2	30 ± 13	23 ± 20.9
BWX-8	164 ± 19.6	215 ± 20.9
BWX-11	150 ± 13	38 ± 0
BWX-17	90 ± 6.5	171 ± 0
BWX-20	20 ± 13	86 ± 2.3
BWX-23	94 ± 13	160 ± 36.6
BWX-24	159±13	289 ± 20.9

Activities are average of duplicate determinations ± SD. Growth conditions: 60^0C, 0.5% (w/v) substrate, pH 7, 120 h; MCC, microcrystalline cellulose; BWX, birchwood xylan

4. Conclusions

In this work, a thermophilic microbial consortium, enriched from yard waste compost, was shown to produce cellulose and xylan degrading enzymes with potential for biomass hydrolysis. The thermophilic microbial consortium was able to adhere to, grow on and hydrolyze lignocellulosic substrates such as corn stover and prairie cord grass as single carbon source. The crude enzyme was active in a wide pH and temperature spectrum with a pH optimum of 4.0 and a temperature optimum of 60^0C (cellulase) and 70^0C (xylanase). The thermophilic enzymes displayed good thermostability with enzyme half lives at 60^oC of 15 h (cellulase) and 18 h (xylanase). The crude enzyme, composed of three cellulase and three xylanase proteins, was 77% more active on insoluble cellulose (avicel) than soluble cellulose (carboxymethyl cellulose) and exhibited substrate specificity towards lignocellulosic substrates such as xylan, cellulose and pine wood. The thermophilic microbial consortium was shown to produce significantly higher hydrolytic activities as compared to the individual cultures isolated from it. This points out to the strong synergistic interactions that exist within the consortium resulting in increased secretion of cellulolytic and xylanolytic enzymes with enhanced hydrolytic potential on lignocellulosic substrates. There appears to have been no prior reports to date on the biochemical and kinetic characterization of cellulose and xylan degrading enzymes from any thermophilic microbial consortium. Furthermore, it was demonstrated that the lignocellulosic hydrolyzates produced with the thermophilic enzymes can be fermented to ethanol. This could have important implications in the enzymatic breakdown of lignocellulosic biomass for the establishment of a robust and cost-efficient process for production of cellulosic ethanol.

Acknowledgements

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Vasudeo Zambare Ph.D, FISBT, is Research Scientist in the Center for Bioprocessing Research & Development, South Dakota School of Mines and Technology, Rapid City, SD, USA. He completed his Ph.D. in Biochemistry from University of Pune, Agharkar Research Institute, India. He is a Fellow of the International Society of Biotechnology in India. Recently, he was honored as associate editor, technical editor, advisory board member and editorial board member for 32 international research journals and as reviewer for 52 international journals. He has expertise in microbial enzymes, extremophiles, renewable energy sources (biomass, biofuel and bioenergy), antimicrobial peptides and biodegradable plastic for biomedical applications. Vasudeo is the author of 3 patents and over 55 peer-reviewed papers, book chapters, conference proceedings, presentations and invited lectures.
E-mail address: Vasudeo.Zambare@sdsmt.edu

Archana Zambare M.S, is Research Assistant in the Center for Bioprocessing Research & Development, South Dakota School of Mines and Technology, Rapid City, SD, USA. She completed her M.S. in Microbiology from North Maharashtra University, Jalgaon, India. She worked as research scholar at Agharkar Research Institute, Pune, India. Her research interests include antioxidant lichens, extremophilic microbes for biofuel and value added biochemical and polymer production. Archana published 2 peer-reviewed journal articles and participated in 2 international conferences.

Kashivishvanath Muthukumarappan Ph.D, is Professor of Food and Bioprocess Engineering in the Department of Agricultural and Biosystems Engineering, South Dakota State University, Brookings, SD, USA. He joined the faculty in 1997 as an Assistant Professor and was promoted to tenured Professor in 2006. He earned his B.S. in Mathematics and Agricultural Engineering in India, a M.S. degree in Food Engineering in Thailand, and completed his Ph.D. in Agricultural Engineering from University of Wisconsin, Madison, USA. He has served as vice chair of the Biomass Energy and Industrial Products committee, and associate editor of Transactions of the ASABE. Kasi's research interests are in food process engineering and in the bioconversion of lignocellulosic biomass into ethanol. He has authored or coauthored more than 90 peer-reviewed publications and made more than 200 regional, national, and international presentations.

Lew Christopher Ph.D., PE, is Director of the Center for Bioprocessing Research & Development and leads a team of more than 120 researchers, graduate and undergraduate students from 8 departments at 2 universities in SD, USA - SD School of Mines and Technology (Rapid City, SD, USA), and SD State University (Bookings, SD, USA). He holds a Masters degree in Chemical Engineering and a PhD degree in Biotechnology and has more than 20 years of industrial and academic experience in the field of bioprocessing of lignocellulosic biomass. His research mission is to add value to the national bioeconomy by applying an integrated biorefinery approach in the development of renewable technologies. He currently serves as member of the editorial board of several international biotechnology journals. Lew's research interests include biomass degradation and conversion for sustainable production of bioenergy and high-value products; enzyme production and catalysis, bioremediation and biorefineries. Lew is the author of 7 patents and over 230 peer-reviewed papers, book/chapters, technical reports, conference proceedings, presentations and invited lectures to Europe, North America, Africa and Asia.

E-mail address: Lew.Christopher@sdsmt.edu