Alkaliphilic Endoxylanase from Lignocellulolytic Microbial Consortium Metagenome for Biobleaching of Eucalyptus Pulp

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Enzymatic pre-bleaching by modification of pulp fibers with xylanases is an attractive approach to reduce the consumption of toxic bleaching chemicals in the paper industry. In this study, an alkaliphilic endoxylanase gene was isolated from metagenomic DNA of a structurally stable thermophilic lignocellulose-degrading microbial consortium using amplification with conserved glycosyl hydrolase family 10 primers and subsequent genome walking. The full-length xylanase showed 78% sequence identity to an endo-β-1,4-xylanase of Clostridium phytofermentans and was expressed in a mature form with an N-terminal His₆ tag fusion in Escherichia coli. The recombinant xylanase Xyn3F was thermotolerant and alkaliphilic, working optimally at 65–70°C with an optimal pH at 9–10 and retaining >80% activity at pH 9, 60°C for 1 h. Xyn3F showed a Vₘₐₓ of 2,327 IU/mg and Kₘ of 3.5 mg/ml on birchwood xylan. Pre-bleaching of industrial eucalyptus pulp with no prior pH adjustment (pH 9) using Xyn3F at 50 IU/g dried pulp led to 4.5–5.1% increase in final pulp brightness and 90.4–102.4% increase in whiteness after a single-step hypochlorite bleaching over the untreated pulp, which allowed at least 20% decrease in hypochlorite consumption to achieve the same final bleaching indices. The alkaliphilic xylanase is promising for application in biobleaching step of kraft and soda pulps with no requirement for pH adjustment, leading to improved economic feasibility of the process.

Keywords: Biobleaching, metagenome, microbial consortium, pulp modification, xylanase

Biobleaching of pulps using xylanases is recognized as the first economically feasible biotechnology-based process in the pulp and paper industries. Pre-bleaching with xylanases reduces the need for toxic bleaching chemicals, and is environmentally and economically advantageous [3]. Enzymatic bleaching relies on the unique specificity of hemicellulases, particularly xylanolytic enzymes, in attacking the hemicellulose component in pulps [4, 13, 27]. Endo-β-xylanases cause partial hydrolysis of xylan, facilitating the removal of the lignin–carbohydrate complex (LCC) generated in the pulping process, which acts as a physical barrier to the entry of bleaching chemicals and thus results in enhanced chemical delignification efficiency [27]. The significant benefits of the enzymatic pretreatment step include higher brightness ceilings and reductions in the amounts of chlorine and bleaching chemicals in the bleaching step, which leads to reductions in chemical waste in bleach plant effluents [28].

Xylanases from many microbial sources have been studied for their application in bleaching of various pulps from wood and agricultural residues, including eucalyptus, wheat straw, and bagasse [8, 9, 24, 41]. The enzymes are either crude enzyme preparations from wild-type bacterial and fungal strains (e.g., Bacillus pumilus [41], Trichoderma sp. [35], and Aspergillus sp. [2, 20, 29]), or purified wild-type or recombinant xylanases from various microbial sources (e.g., A. fischeri [32], A. caesipitus [31], and A. fumigatus [17]). The desirable characteristics of xylanases for the bleaching step include the ability to work in an alkaline pH range at relatively high temperature and the lack of cellulase activity [3]. However, most microbial xylanases work optimally in the acidic to neutral pH range, whereas a limited number of xylanases working under alkaline conditions have been reported, mostly from Bacillus strains.
community was obtained, as indicated by denaturing gradient gel electrophoresis (DGGE) analysis. The microbial consortium was maintained by continual subcultivations as described above, or in PCS medium containing 20% glycerol at ~80°C for long-term storage.

**Amplification of xyn3F Gene**

Total genomic DNA was extracted from the microbial consortium using the benzyl chloride method [42]. The purified DNA was used as the template for amplification of the partial xylanase gene using glycosyl hydrolase family 10 conserved primers XYNFR (5'-TMGTTKACMACRTCCCA-3') and XyIF23 (5'-MGNGGICAY ACNYTGTITGCGA-3') with DxyNzyme EXT DNA polymerase (Finzyme, Espoo, Finland), according to the manufacturer’s protocol, with the following PCR conditions: pre-denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 66°C for 1 min, and extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The GenomeWalker library (Clontech, Mountain View, CA, USA) was constructed using the EcoRV-digested genomic DNA ligated to the GenomeWalker adapter and used as the template for amplification of the 5' and 3' fragments. The full-length gene (xyn3F) was then amplified according to the assembled contig sequences using primers XYN3F_F2 (5'-GGCATATGCCTATGCTACATAAGGGTGCCAAAACA-3') and XYN3F_R2 (5'-GCCGAATCTTACGTGATCGTAAACCGAGCATCTG-3') (restriction enzyme recognition sites are underlined). The PCR product was gel-purified using the QIAagen Gel Extraction kit, ligated to pGEM-T vector (Promega, Madison, WI, USA), and transformed into *Escherichia coli* DH5α by the heat-shock method. Transformants were selected on Luria–Bertani agar plates containing ampicillin (100 µg/ml), supplemented with 40 µg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-GAL) and 20 µg/ml isopropyl-β-D-thiogalactopyranoside (IPTG). The full-length xyn3F gene sequence was analyzed (1st Base, Malaysia) and deposited in GenBank under the accession number JX455051.

**Expression and Purification of Xylanase Xyn3F**

The xyn3F gene with no signal peptide sequence was amplified by PCR using the recombinant pGEM-T vector as a template using primers XYN3F_F3 (5'-GGCATATGCCTACATTAAAGGGTGCCAAAACA-3') and XYN3F_R2, incorporated with the NdeI and a BamHI restriction site on the forward and reverse primers, respectively. The gene fragment was digested with the respective restriction enzymes and ligated with pET28a(+) (Invitrogen, Grand Island, NY, USA) digested with the same restriction enzymes. The ligation mixture was transformed into *E. coli* DH5α using the heat-shock method. The recombinant plasmid obtained from the transformants was subjected to DNA sequencing to verify the sequence. The plasmid designated pET-xyn3F containing the xyn3F gene with the upstream His, tag sequence fusion was transformed into *E. coli* Rosetta-gami (DE3)pLysS and selected on LB agar containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol.

The recombinant clone was cultured in LB medium containing the same antibiotics at 37°C with shaking at 200 rpm for 18 h and inoculated into a fresh medium (1% inoculation) and further incubated until the OD₆₀₀ reached 0.6. The culture was induced by addition of 0.5 mM IPTG and incubated at 30°C for 3 h. Cells were harvested by centrifugation at 6,000 × g at 4°C for 15 min and resuspended in lysis buffer (50 mM sodium phosphate buffer, pH 7.5, 5% glycerol, and 0.1% sarcosine). The target enzyme Xyn3F was purified from

**Materials and Methods**

**Preparation of Lignocellulolytic Microbial Consortium**

The lignocellulose-degrading microbial consortium was constructed from microflora originated from high temperature sugarcane bagasse compost as previously described [40]. Briefly, the consortium was constructed by inoculating the compost sample taken at the compost as previously described [40]. Briefly, the consortium was constructed by successive subcultivations in a synthetic medium containing sugarcane bagasse as the carbon source [40]. The microbial consortium efficiently degraded a range of lignocellulosic substrates including plant biomass and pulp waste. The consortium comprised mainly cellulosytic clostridia in stable co-existence with a non-cellulosytic Betaproteobacterium and several *Bacillus*-related strains. Proteomic analysis revealed the complex composition of cellulosytic and hemicellulolytic enzymes in the crude cellulose-bound enzyme fraction, suggesting the potential of the microbial consortium as a promising source for plant biomass-degrading enzymes discovery. In this study, an alkalophilic xylanase gene was isolated from the metagenome of the lignocellulolytic consortium using a conserved gene sequence-based approach. The recombinant xylanase was characterized and applied for pre-bleaching of eucalyptus pulp. The enzyme is promising for biobleaching of kraft and soda pulps with no prior pH adjustment, which is advantageous in reducing chemical consumption in the bleaching step and for improvement in the overall cost efficiency in the pulp industry.

**[7, 11, 34]. Most commercial xylanases for biobleaching also have maximal activity in the neutral pH range, which led to the necessity in pH adjustment of the pulps in the bleaching process, resulting in an additional cost in pulp production. Searching for an alkalophilic xylanase for the biobleaching process is thus of interest in developing a greener and more economically efficient pulp and paper-making process.**

Culture-independent metagenomic gene discovery allows exploration of genetic resources from uncultured microorganisms, which constitutes the majority of microbial populations in environments [6]. Many plant-fiber-degrading enzymes have been identified from uncultured microorganisms from various ecological niches active in lignocellulose degradation (e.g., soil, termite gut, and cow rumen) [33, 37, 38]. Recently, a structurally stable microbial consortium, designated MC3F with high cellulose-degrading activity was constructed by successive subcultivations in a synthetic medium containing sugarcane bagasse as the carbon source [40]. The microbial consortium efficiently degraded a range of lignocellulosic substrates including plant biomass and pulp waste. The consortium comprised mainly cellulosytic clostridia in stable co-existence with a non-cellulosytic Betaproteobacterium and several *Bacillus*-related strains. Proteomic analysis revealed the complex composition of cellulosytic and hemicellulolytic enzymes in the crude cellulose-bound enzyme fraction, suggesting the potential of the microbial consortium as a promising source for plant biomass-degrading enzymes discovery. In this study, an alkalophilic xylanase gene was isolated from the metagenome of the lignocellulolytic consortium using a conserved gene sequence-based approach. The recombinant xylanase was characterized and applied for pre-bleaching of eucalyptus pulp. The enzyme is promising for biobleaching of kraft and soda pulps with no prior pH adjustment, which is advantageous in reducing chemical consumption in the bleaching step and for improvement in the overall cost efficiency in the pulp industry.
the crude soluble fraction using a HisTrap column (GE Healthcare Biosciences, Pittsburgh, PA, USA) following the manufacturer’s protocol. The enzyme was further purified to high homogeneity using a Superdex G75 gel filtration column (GE Healthcare Biosciences) using 50 mM sodium phosphate buffer, pH 7.4, 0.15 M NaCl as the mobile phase. The purified enzyme was analyzed on 12% SDS-PAGE gels and visualized by staining with Coomassie Blue G250. The pooled fractions with xylanolytic activity was concentrated and desalted on an Amicon Ultrafiltration unit (Millipore, Billerica, MA, USA). The concentration of the enzyme was determined by the Bradford assay (Bio-Rad, Hercules, CA, USA) using bovine serum albumin as a standard.

**Enzyme Activity Assay**

Xylan degrading activity was analyzed based on the amount of liberated reducing sugars by the 3,5-dinitrosalisylic acid (DNS) method [25] as previously described [30]. The 200 μl standard assay reactions contained appropriate dilutions of the enzyme in 100 mM Tris-HCl buffer, pH 9.0 and 1% (w/v) of birchwood xylan as the substrate. The reactions were incubated at 70°C for 10 min. Other polysaccharide degrading activities were analyzed by the DNS method as described using carboxymethyl cellulose for CMCase activity, Avicel for cellulase activity as exoglucanase, and filter paper for FPase activity. The amount of reducing sugars was determined by absorbance measurements based on standard curves prepared using the corresponding sugars. The β-xylosidase activity was assayed using p-nitrophenyl xylopyranoside at 70°C in 100 mM Tris-HCl buffer, pH 9.0. Kinetic parameters were analyzed using birchwood xylan as a substrate under the standard conditions. One international unit (IU) was defined as the amount of enzyme liberating 1 μmole of reducing sugars or the corresponding products per minute under the assay conditions. Experiments were done in triplicate, and the standard deviation was < 5%.

**Pulp Bleaching**

Eucalyptus soda pulp was obtained from a pulp and paper mill in Thailand. The unbleached pulps after the oxygen delignification step had a kappa number of 14.3. All biobleaching experiments were carried out using 15 g (dried weight basis) of eucalyptus pulp at 10% consistency with 50 IU/g of Xyn3F incubated at 60°C for 1 h with no prior pH adjustment (approx. pH = 9.2) in polyethylene bags. The pulps at 10% consistency were bleached with 0.24–0.30% sodium hypochlorite in the presence of 0.1% (w/v) sodium hydroxide and incubated at 50°C for 3 h. Control (with no enzyme) reactions were included. The pulp was washed with reverse-osmosis purified water after each stage of bleaching.

**Analysis of Pulp Physical Properties**

Paper sheets of bleached pulps were prepared according to the TAPPI method T272 om-92. Brightness measurements of the paper sheets were made on a Technibrite Micro TB-1C (Technidyne, USA), by the ISO 2470 TAPPI standard method, 1996. The color of paper sheets was determined with a spectrophotometer (UltraScan XE, HunterLab, USA), using CIE L*a*b* notation. The instrument was set for a 10 degree observer, the small area of view (1/4 inch diameter) was used, and specular reflectance was included. Spectra were recorded from 375 to 750 nm, using the visible light source, at three different locations on each side of each paper sheet sample. The triplicate readings were averaged. All values reported, representing an average of three separate measurements, had a standard deviation less than 5%.

**Scanning Electron Microscopy Analysis**

The structure and morphology of the pulp fibers were analyzed by scanning electron microscopy (SEM) using a VP-SEM S-3400N and EDX Scanning Electron Microscope (Hitachi, Krefeld, Germany). The samples were dried and coated with gold for analysis. An electron beam energy of 10 kV was used for the analysis.

**RESULTS AND DISCUSSION**

The lignocellulolytic consortium MC3F is a structurally stable lignocellulose-degrading microbial community, growing optimally at 50°C under slightly alkaline conditions [40]. The consortium comprised microaerobic and facultative anaerobic bacteria (a Betaproteobacterium HMD44 with 100% identity to Rhodocyclaceae sp.) that coexisted with strictly anaerobic cellulolytic bacteria closely related to Clostridium sp. strains Z6, dgC140, and J4, and Thermoaerobacterium thermosacharolyticum strain W16, in addition to a few minor bacilli and unidentified uncultured bacteria. Its high structural and functional stabilities allow application of the consortium for direct lignocellulose degradation and as a source of industrially potent plant polysaccharide-degrading enzymes. Proteomic analysis of the cellulose-bound fraction of crude MC3F enzyme showed the presence of diverse lignocellulose-degrading enzymes of clostridia and bacilli origins in different glycosyl hydrolyase families (GH8, 9, 10, 16, and 48) either as free enzymes or cellulosomal components. The cloning of a GH10 endoxylanase from MC3F as identified by LC/MS-MS was the focus of this study, aiming for searching a potent enzyme for application in the biobleaching step in the pulp industry.

**Cloning of xyn3F Gene**

Culture-independent enzyme screening with conserved GH10 primers led to amplification of a partial xylanase gene fragment (0.16 kb). The amplified sequence was cloned into pGEM-T for initial sequence analysis. Specific primers were designed to obtain the upstream and downstream gene fragments by genome walking. The full-length gene (xyn3F) covering an open reading frame containing 1,587 bp encoding a putative xylanase was then amplified according to the assembled contig sequences by nested PCR. DNA sequence analysis revealed that the putative gene encoded a GH10 endoxylanase gene fragment with 78% and 64% sequence identity to that of C. phytofermentans ISDg and C. lentocellum DSM5427, respectively. C. phytofermentans is a strictly anaerobic Gram-positive bacterium with the distinct ability of direct degradation and conversion of lignocellulosic biomass to biofuels, especially ethanol and hydrogen [39].
The deduced amino acid sequence of Xyn3F (528 amino acid residues) contained a glycosyl hydrolase family 10 conserved motif (positions 441-451). Based on multiple sequence alignment, Glu336 and Glu448 were assigned as the putative catalytic sites for Xyn3F. The enzyme has a theoretical Mw and pI of 58,718 Da and 8.85, respectively.

**Heterologous Expression and Purification of Xyn3F**

The DNA fragment encoding the mature Xyn3F with no signal peptide (1,494 bps) was amplified and ligated with pET28-a(+). The recombinant plasmid was designated pET-xyn3F and transformed into the expression host *E. coli* Rosetta-gami (DE3)(pLysS). An induced protein band was observed after induction of the recombinant *E. coli* containing pET-xyn3F by IPTG (Fig. 1). The induced protein was mainly expressed in the soluble fraction and recognized by anti-His<sub>6</sub> antibody. The His<sub>6</sub> fusion protein was then purified using a HisTrap column. The target protein fraction was eluted with 100 mM imidazole and concentrated by ultrafiltration. The active fractions were further purified to high homogeneity (>95%) using a Superdex G75 gel filtration column. The two-step purification resulted in a target enzyme yield of 40.09% and purification fold of 10.9 (Table 1).

**Biochemical Characterization of Xyn3F**

Biochemical characterization revealed that the enzyme worked optimally at 70°C with an optimal pH at 9.0 (Fig. 2A). The enzyme retained >70% activity after pre-incubation at pH 7–9 for 5 h at 25°C and showed 87.0% remaining activity after incubation at 60°C, pH 9.0, for 60 min (Fig. 2B). Although Xyn3F showed optimal activity at 70°C, no residual activity was observed when the enzyme was pre-incubated at this temperature for 1 h in the absence of the substrate. The result thus suggested enzyme stabilization effects by the polysaccharide substrate, which led to improved enzyme performance in reactions at high temperature [36]. The enzyme showed higher specificities toward hydrolysis of birchwood xylan, with the specific activity of 1,794.3 IU/mg equivalent to the volumetric activity of 71.13 IU/ml. Lower activity was observed on beechwood xylan with a slight activity on carboxymethyl cellulose. No activity was detected on microcrystalline cellulose, filter paper, and p-nitrophenyl sugar substrates, indicating endo-β-1,4 xylanolytic activity as the major activity of Xyn3F (Table 2). The enzyme kinetics of recombinant Xyn3F against birchwood xylan was determined at the optimal conditions (70°C and pH 9.0). The K<sub>m</sub>, V<sub>max</sub>, and k<sub>cat</sub> values calculated from the hyperbolic curve according to the enzyme kinetic equation of Michaelis–Menten were 3.5 mg/ml, 2,327 µmol/mg/min, and 2,133.08 s<sup>-1</sup>, respectively.

Xyn3F showed optimal activity under relatively high pH and temperature conditions when compared with most previously reported xylanases from different microbial sources, including those from fungal (*e.g.*, *Aspergilli*, *Trichoderma*, and *Penicillium* [10, 19, 21, 35]) and bacterial origins (*e.g.*, *Acidobacterium* and *Cryptococcus* [15, 16]), which tended to be acidophilic or showed lower optimal pH and less optimal temperature. The enzyme showed high alkaliphilicity in the high temperature range for biobleaching compared with many alkaliphilic xylanases from *Bacillus* sp. JB99, *B. pumilus* 13a, *B. halodurans* S7, *Gracilibacillus* sp. TSCPVG, and *Konuria* sp. Mn22, which showed pH and temperature optima in the range of 7.5–9.0 and 55–70°C, respectively [7, 12, 22–24]. Alkaliphilicity and stability of xylanases have been proposed to be related to similar factors contributing to thermostability [14]. In particular, high pH optima of
Xylanases may be related to several factors such as increases in the overall pKa of the catalytic site residues or an increase in positively charged residues (e.g., asparagine or arginine) particularly on the enzyme surface, which make the enzyme more resistant to the structural changes and catalytic deactivation at high pHs [5], although a contrast finding in correlation of high acidic/basic amino acid residue ratio to alkaliphilicity was reported for GH10 xylanases [23]. As Xyn3F worked optimally in the alkaline pH range at relatively high temperature, the potential of using the enzyme in the biobleaching step in the pulp industry is thus of interest.

**Application Testing of Xyn3F in Biobleaching**

The potential of using Xyn3F as a pulp bleaching enhancer was preliminarily evaluated by analyzing the hydrolysis activity of HisTrap-purified enzyme on industrial eucalyptus pulps. The pre-bleaching reaction was performed at 10% (w/v) pulp consistency, using 5, 10, and 50 U of enzyme/g moisture-free pulp and the amount of reducing sugar released was determined. The result showed that increasing reducing sugars was found with increasing enzyme concentrations. The highest reducing sugar was obtained at 50 IU of enzyme/g pulp (Fig. 3). The purified Xyn3F at the 50 IU/g dosage was then used to study its ability in the pulp bleaching process.

The pulp bleaching efficiency of the enzyme was assessed based on its effects on pulp brightness, whiteness, and color indices using the simplified one-step hypochlorite bleaching at varying hypochlorite concentrations. In all conditions examined, pretreatment of the eucalyptus pulps with crude or purified Xyn3F showed an increase in the key pulp bleaching indices (Fig. 4). The brightness and whiteness of the xylanase pulps were improved to 51.0% ISO and -9.26 CIE, respectively, equivalent to

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**Table 2. Substrate specificities of Xyn3F.**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Specific activity (U/mg)</th>
</tr>
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<tbody>
<tr>
<td>Birchwood xylan</td>
<td>1,794.4 ± 136.6</td>
</tr>
<tr>
<td>Beechwood xylan</td>
<td>1,029.5 ± 55.2</td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>52.5 ± 0.1</td>
</tr>
<tr>
<td>Avicel</td>
<td>ND¹</td>
</tr>
<tr>
<td>Filter paper</td>
<td>ND¹</td>
</tr>
<tr>
<td>p-Nitrophenyl-β-D-xylopyranoside</td>
<td>ND¹</td>
</tr>
<tr>
<td>p-Nitrophenyl-β-D-arabinopyranoside</td>
<td>ND¹</td>
</tr>
</tbody>
</table>

¹ND: no detectable activity.

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**Fig. 2.** Effects of temperature and pH on the activity and stability of Xyn3F.

(A) Effects of temperature: The reactions contained 1% (w/v) of birchwood xylan in 100 mM Tris-HCl buffer, pH 9.0, and were incubated at the corresponding temperatures for 10 min for optimal temperature study. For thermostability study, Xyn3F was pre-incubated in 100 mM Tris-HCl buffer, pH 9.0, at different temperatures for 1 h before residual activity analysis under the standard assay conditions. (B) Optimal pH: The reactions were assayed in 50 mM sodium acetate (pH 3–5), sodium phosphate (pH 6–9), or Tris-HCl (pH 8–10) buffer at 70°C for 10 min. (C) pH stability: Xyn3F was pre-incubated in 100 mM sodium phosphate (pH 7–8) or Tris-HCl (pH 8–9) buffer at 25°C for 1–5 h before residual activity analysis under the standard assay conditions.

**Fig. 3.** Hydrolysis of eucalyptus pulp by Xyn3F.

Reactions contained 10% (w/v) eucalyptus pulp with 5–50 IU/g pulp of HisTrap-purified enzyme and were incubated with no prior pH adjustment (pH 9.2) at 60°C for 1 h.
6.69% and 49.48% increases over the untreated pulp. Further increases in pulp bleaching indices were observed after the subsequent hypochlorite bleaching step in which 4.49% and 102.40% increases in brightness and whiteness indices, respectively, were obtained at 0.30% hypochlorite compared with the respective control with no enzyme treatment. Only slight decreases in the bleaching indices were observed on the resultant enzyme-treated pulps at decreasing hypochlorite concentrations. Overall, treatment of eucalyptus pulp with Xyn3F under the experimental conditions led to substantial increases in the key pulp bleaching indices at all hypochlorite concentrations, equivalent to 4.49–5.07% and 90.44–102.40% improvements in pulp brightness and whiteness, respectively. A similar trend in improvement in pulp bleaching susceptibility was observed by analysis of Lab color space, which is a three-dimensional color-opponent space used for defining perceived color. A substantial increasing lightness index ($L^*$) was obtained for the enzyme-treated pulps, whereas only slight changes in color indices ($a^*$ and $b^*$) were found for the enzyme treated pulps with the small shifts towards the blue and green colors in the resultant pulps (Table 3). The results thus indicate that at least 20% hypochlorite consumption could be reduced by the enzymatic pretreatment step in order to reach the final pulp brightness of the control from the conventional H-stage bleaching. The reduction in hypochlorite dosage was comparable to other previous work using hypochlorite or other bleaching agents, in which 10% of hypochlorite or 20% of organochlorine could be reduced by the enzymatic pretreatment step [20, 26].

**Scanning Electron Microscopy Analysis of Xyn3F-Pretreated Pulp**

Physical changes in xylanase-pretreated pulps were studied using scanning electron microscopy (Fig. 5). The native pulp fibers were smooth and relatively intact, with no fibrillation. After Xyn3F treatment, the pulp fiber surface became rough and heterogeneous owing to the peeling effects of the enzyme, similar to that observed for other...
enzyme pretreated pulps using xylanases from various microbial sources on pulp from different origins [18]. Subsequent hypochlorite bleaching led to swelling and loss in compactness of the fibers, with increases in fibrillation, particularly for the enzyme-treated fibers compared with the controls. This thus suggested the effects of Xyn3F on partial hydrolysis of the xylan components in pulps, which introduced greater porosity and facilitated an increase in fibrillation of the resultant fibers [1]. These surface modifications resulted in increasing pulp accessibility by the bleaching chemical and decreasing diffusion resistance on removal of degraded lignin from pulps. The observed morphological changes in the pulp fibers thus indicate the efficiencies of Xyn3F as a bleaching enhancer under the experimental conditions in this study.

In conclusion, an alkaliphilic and thermophilic xylanase originated from a lignocellulolytic microbial consortium metagenome has been reported in this study. The enzyme represented the first alkaliphilic xylanase from clostridia. The potential of the enzyme for pre-bleaching of eucalyptus pulps has been demonstrated, where it reduced the bleaching chemical dosage with the advantage of no necessity for prior pulp pH adjustment. Application of this enzyme is thus feasible for developing a greener and more economically attractive pulp bleaching process in the industry.

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