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Bacterial Strain for Bast Fiber Crops Degumming and Its Bio-Degumming Technique

Shengwen Duan
Institute of Bast Fiber Crops, Chinese Academy of Agriculture Sciences
Bingrong Xu
Huzhou Nanxun Shanlian Shengye Textile Co., Ltd
Lifeng Cheng
Institute of Bast Fiber Crops, Chinese Academy of Agriculture Sciences
Xiangyuan Feng
Institute of Bast Fiber Crops, Chinese Academy of Agriculture Sciences
Qi Yang
Institute of Bast Fiber Crops, Chinese Academy of Agriculture Sciences
Ke Zheng
Institute of Bast Fiber Crops, Chinese Academy of Agriculture Sciences
Mingqiang Gao
Institute of Bast Fiber Crops, Chinese Academy of Agriculture Sciences
Yuande Peng (343778471@qq.com)
Institute of Bast Fiber Crops, Chinese Academy of Agriculture Sciences

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Abstract

The R&D of bio-degumming technology is under a slow progress due to the shortage of proper efficient bacterial strains and processes. A degumming bacterial strain—*Pectobacterium wasabiae* (PW)—with broad-spectrum degumming abilities was screened out in this study. After the fermentation for 12 h, the residual gum contents of kenaf bast, ramie bast, hemp bast, flax bast, and *Apocynum venetum* bast were all lower than 15%. This bacterial strain could realize the simultaneous extracellular secretion of pectinase, mannase, and xylanase with the maximum enzyme activity levels of 130.25, 157.58, and 115.24 IU/mL, respectively. The optimal degumming conditions of this bacterial strain were as follows: degumming time of 12 h, bath ratio of 1:10, temperature of 33 ℃, and inoculum size of 2%. After the bio-degumming through this bacterial strain, the COD in wastewater was below 4,000 mg/L, which was over 60% lower than that in boiling-off wastewater generated by chemical degumming. This technology achieves higher efficiency, higher quality, and lower pollution.

Introduction

The improvement in human living quality and the shortage of petroleum and forest resources have encouraged various countries in the world to seek for new-type natural plant fiber resources. Cellulose is a renewable natural polymer resource that is rich in nature, and it is a cheap and inexhaustible resource. Among all kinds of plant fiber resources, bast fiber plants such as ramie, kenaf, and industrial hemp have fast growth, high yield, strong adaptability, and environmental protection. Bast fibers are of irreplaceable important development values by virtue of antibiosis, insect prevention, breathable moisture absorption, and natural degradation (Xiong, 2008; Subasinghe et al.; Crini and Lichtfouse, 2020).

The chemical degumming method centering on soda cooking, which is commonly used at home (China) and abroad, is a process that aims to acquire cellulosic fiber satisfying the follow-up processing requirements by catalyzing the degradation of bast non-cellulosic substances under acidic and high-temperature conditions with some aftertreatment measures. This process not only seriously pollutes the environment and damages the fibrous quality but also has high processing cost; these limitations restrict its industrial development (Liu, 2013; Fan, 2015). Bio-degumming is a process that centers on catalyzing the degradation of non-cellulosic substances by biocatalysts (enzymes) and aims to obtain the cellulosic fiber satisfying the follow-up processing requirements. Bio-degumming is a cyclic action process of “enzyme producing strains–enzyme degumming–gum culturing strain,” and it is a multienzyme collaborative catalytic system in living organism in essence. The bast fiber crop bio-degumming technology, which has low energy consumption, low pollution, low cost, and high quality, can overcome the drawbacks of conventional degumming method; it is also the development direction of the bast fiber crop processing industry (Liu, 2009; Cheng, 2011; Fan et al., 2015).

Bast non-cellulosic substances have extremely complicated composition and structure, and the non-cellulosic degradation can be realized only through the collaborative action of key enzymes such as pectinase (Zhou et al., 2015; Zhou et al., 2017), mannase (Wang et al., 2017), xylanase (Biswas et al., 2016), and lignin-degrading enzyme (Ding et al., 2014; Yang, 2016). The bio-degumming technology has been studied for over 50 years at home and abroad, but the large-scale productivity has not been formed yet. The primary reason is that no suitable bio-degumming strains have been bred, and this situation is the bottleneck of bast fiber crop bio-degumming technology. At present, very few high-efficiency bast fiber crop degumming strains have been reported (Basu et al., 2009; Duan et et al., 2012; Tong et al., 2020; Duan et et al., 2018).

A plant putrefying bacterial strain—*Pectobacterium wasabiae* (PW) (preservation number: CGMCC 14601), which could efficiently degrade ramie colloids to extract ramie fibers, was reported in this study. This bacterial strain is characterized by complete enzyme system, high enzymatic activity, fast reproduction, low residual gum content after ramie degumming, and no production of cellulase. Thus, it shows favorable degumming effects on ramie, kenaf, and industrial hemp. Moreover, a set of optimized ramie bio-degumming processing technology has been formed and put under pilot-scale test in the enterprise for 1 month and has a good industrial prospect.

Materials And Methods

Raw materials

Ramie bast: purchased from Yuanjiang City, Hunan Province, China. The ramie bast was dried and shell-less without mildew.

Kenaf bast: Hunan No.1 kenaf planted in Changsha.

Industrial hemp bast: purchased from Sunwu County, Heilongjiang Province, China.

Bacterial strain, classification, and determination

The bacterial strain PW was screened out by the research group.

The morphological and physiological–biochemical characteristics of this bacterial strain were analyzed in accordance with *Bergey's Manual of Determinative Bacteriology (9th edition)*. The 16S rRNA gene of strain PW was amplified with the universal primer pairs 27 F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492 R (5'-TACGGCTACCTTGTTACGACT-3') and sequenced by Shanghai Sangon Biotech Company. 16S rRNA genes were used to construct the phylogenetic tree used MEGA by the minimum-evolution method and the neighbor-joining method at the parameter of 10 Program bootstrap values based on 1,000 replications.

Strain culturing

The PW strain was cultured at 34 ℃ and a rate of 120 rpm for 8 h. The formula of culture medium was as follows: glucose (1.0%), NaCl (0.5%), beef extract (0.5%), peptone (0.5%), and pH (6.5–7.0).

2.4 Optimization of bio-degumming conditions

Mildew-free kenaf bast was collected and exposed to the sun for 1–2 days. The head and tail parts were clipped by 20 cm, and the sample was then shaken to remove the dust. With different conditions (temperature: 31–37 ℃, bath ratio: 1:10–1:25, fermentation time: 5–20 h, and inoculum size: 1%–4%) set, the degumming was conducted by means of oscillating fermentation on a shaking table at a rate of 180 rpm, and different orthogonal test factors and levels (Table 1) were set. The orthogonal data were statistically analyzed via IBM SPSS 22.0 software. After the degumming was completed, the sample was immediately boiled in 100 ℃ hot water for 20 min and then washed in a bast fiber crop washing machine.

Table 1 Orthogonal test factors and level of fermentation parameters

	A	B	C	D
Level	Inoculum	size	(%)	Temperature
1	1.0	27	1:10	8
2	1.5	29	1:15	12
3	2.0	31	1:20	16
4	2.5	33	1:25	20

Comparative degumming test

The comparative degumming tests of ramie, kenaf, and industrial hemp were conducted using the chemical degumming method, water retting degumming method, and optimized bio-degumming process. The technical routes were as follows:

(1) Technical route of bio-degumming:

Bast fiber crop→ pretreatment→ inoculation →soaking and fermentation→ inactivation (NaOH mass concentration: 0.5 g/L) → washing→ dewatering→ drying→ bast fiber.

(2) Technical route of chemical degumming:

Bast fiber crop→ pretreatment→ acid dipping (1 mL/L, 50 ℃, bath ratio: 1:10, 60 min) → washing → primary soda boiling by pressurization (NaOH mass concentration: 8 g/L, $Na_5P_3O_{10}$ 2.5g/L, Na_2SiO_3 2 g/L, batch ratio: 1:10, 1.5 h)→ washing →fiber beating→ dewatering→ secondary soda boiling (NaOH mass concentration:12 g/L, $Na_5P_3O_{10}$ 2 g/L, Na_2SiO_3 2 g/L, bath ratio: 1:10, 2.5 h)→ fiber beating→ dewatering→ washing→ dewatering→ drying→ bast fiber. (Shao, 2003; Deng, 2010)

(3) Technical route of water retting degumming:

Bast fiber crop→ natural water soaking→ fermentation→ washing→ drying→ bast fiber.

Degumming effect test

(1) Non-fiber removal rate

Weight loss ratio: Constant-weight bast fiber crop (M0) was acquired after drying and before degumming, and it was washed after the fermentation and dried to constant weight (M1). Weight loss ratio V= $(M_0 - M_1)/M_0 \times 100$ % (Zeng et al., 2007).

The residual gum content was detected by referencing to the quantitative analysis method of ramie chemical components (Jiang and Shao, 2005).

(2) COD detection: The COD detection in the fermentation broth after degumming was conducted via COD detector ET99718 (Lovibond® Group, Germany) by following the specifications.

Enzymatic activity in the degumming process

The enzymatic activity was defined as the enzyme dosage needed to degrade 1 μmol of substrate per min. The dinitrosalicylic acid (DNS) method was used to detect the enzymatic activity of the bacterial strain (Do et al., 2016; Wang, 2009).

Monosaccharide detection in the degumming process

From 0 h when the bast fiber crop was inoculated and oscillated until 20 h when the degumming was completed, the fermentation broth sample was collected every other 4 h and separated using 0.2 μm film coating (Vivaflow 200). The filtered solution was used to detect the monosaccharide content. The monosaccharide components generated after the bast fiber crop hydrolysis were systematically analyzed through 1-phenyl-3-methyl-5-pyrazolone precolumn derivatization high-performance liquid chromatography (HPLC) method. After the bast fiber was hydrolyzed using trifluoroacetic acid, C_{18} chromatographic analysis column and UV detector were used to detect the monosaccharides in the hydrolysate and the components and contents of their derivatives (Zhang et al., 2013; Fang et al., 2015).

Degumming fiber detection

After degumming for 8 m, slices were made and observed under an electron microscope JEOL-1230 (JEOL Ltd., Japan) through thousand-fold amplification. The fibers were observed under KH-2700 3D video microscope by 100-fold amplification.

Results And Discussion

Morphological and physiological–biochemical characteristics of bacterial strain PW

After activated-state bacterial solution grew in the broth medium for 8 days, the bacterial strain was rod-shaped without spores, and the average size was 0.6 μm×1.5 μm (Fig. 1a). After growing on the broth plate for 20 h, PW became round, white, and humid with micro-bulges and transparent edges (Fig. 1b). Gram-negative result was manifested through the starch and urease tests, and positive results appeared in the casein, catalase, indole, and nitrate reduction tests.

The 16S rRNA gene sequence of PW was a nucleotide sequence with the full length of 1375bp, and it was submitted onto GenBank to acquire accession number GU097456. On the basis of the 16S rRNA gene sequence of PW, the minimum-evolution method was used to construct the phylogeny tree diagram (Fig. 2). Meanwhile, another phylogeny tree diagram was established through the neighbor-joining method, and the results were consistent in essence. The results showed that the bacterial strain PW belonged to *Pectobacterium* sp. and had a high similarity to 16S rRNA gene sequence of *Pectobacterium wasabiae* (NR 026047). By combining the physiological and biochemical characteristics, PW was identified as *Pectobacterium wasabiae*, which was preserved in China General Microbiological Culture Collection Center (CGMCC), with the preservation number of CGMCC No. 14601.

Among the already reported degumming bacterial strains, germs account for a large proportion, and most belong to *Bacillus* and *Pectobacterium*, including *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus tequilensis* (Yang et al., 2018; Basu et al.), *Bacillus aryabhattai*, *Bacillus thuringiensis*, *Lysinibacillus fusiformis*, *Acidovorax temperans* (Cheng et al., 2020; Chiliveri et al., 2016; Zheng et al., 2001), and *P. carotovorum*, *Pectobacterium chrysanthemi* (Shu et al., 2020; Duan et al., 2016; Duan et al., 2018). The bacterial strain reported in this study also belongs to *Pectobacterium sp.* In the microbial screening of bast fiber crop degumming, *Bacillus sp*. and *Pectobacterium sp*. show outstanding performance and are thus worthy of attention.

Bio-degumming fermentation parameters

After the treatment of kenaf bast under different fermentation conditions, the test results of raw material weight loss ratio are shown in Table, and the analysis of variance (ANOVA) and multiple comparisons results are listed in Tables 3 and 4. According to the ANOVA results, the bath ratio and time significantly influenced kenaf weight loss ratio, which was insignificantly affected by the inoculum size or temperature. From the table of multiple comparisons, the influencing degrees of inoculum size, temperature, bath ratio, and time on kenaf weight loss ratio were sorted in a descending order as time, bath ratio, temperature and inoculum size. Their minimum influencing degrees on kenaf weight loss ratio were the optimal levels. Thus, the combinational optimal levels were obtained as follows: time of 12 h, bath ratio of 1:10, temperature of 33 ℃, and inoculum size of 2%.

In contrast to the traditional water retting degumming and rain & dew degumming, one of important features of bio-degumming is short degumming time. The traditional water retting degumming of ramie and kenaf and rain & dew degumming of hemp and flax generally take 7–30 days, and they are greatly affected by the external environmental and climatic conditions (Liu and sun, 2018; Zhan, 2005). However, the bio-degumming can complete the degumming process within 1 day, and the bacterial strain can finish the degumming of kenaf bast within 12 h.

Table 2 Loss rate of kenaf bast with different fermentation conditions

Sample name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Weight loss ratio of raw jute (%)	15.6	19.2	20.1	17.8	20.6	15.2	16.9	17.5	17.9	20.4	14.8	14.8	19.6	16.5	18.4	15.9

Table 3 Variance analysis of bio-degumming fermentation parameters

	F	P
inoculum size	0.540	0.687
temperature	6.193	0.084
bath ratio	9.402	0.049
time	9.757	0.047

Table 4 Multiple comparison of the parameters for kenaf bio-degumming

	inoculum size	temperature	bath ratio	time
M1	17.435	18.9675	15.5175	17.835
M2	17.7175	17.8175	17.935	15.6675
M3	17	16.935	17.435	19.2675
M4	17.7675	16.2	19.0325	17.15
N	0.7175	2.7675	3.515	3.6

Note: M represents the index level, M1–M4 denote the 1^{st}–4^{th} levels, and N is the statistical magnitude, which manifests the influence of this factor on the raw material weight loss ratio.

Enzymatic activity in fermentation broth (unit: U/mL)

Pectinase, mannase, and xylanase could be detected in the whole degumming process; the pectinase activity during the process was increased from 12.52 IU/mL at 0 h to 130.25 IU/mL at 15 h, mannase activity was from 14.82 IU/mL to 157.58 IU/mL, and xylanase activity was from 11.74 IU/mL to 115.24 IU/mL; the growth rates were high within 0–9 h and slowed down after 12 h, but they still kept growing (Table 5).

Table 5 Activity of enzymes secreted by strain PW during degumming (unit: U/mL)

	Fermentation time (h)					
	0	3	6	9	12	15
Pectinase	12.52	23.21	57.54	101.47	121.22	130.25
Mannase	14.82	35.66	74.25	118.24	145.56	157.58
Xylanase	11.74	25.56	45.58	86.52	106.75	115.24

Bast fiber crop, which is composed of complicated components, generally contains 4%–8% of pectin, 12%–18% of hemicellulose, and 2%–5% of lignin. During the microbial degumming process, the degumming bacterial strain realizes the growth and proliferation through the nutrient substances in the degumming solution and secretes extracellular enzyme systems such as pectinase. It also decomposes macromolecular pectin substances into micromolecular substances, absorb them *in vivo*, and transforms them into soluble micromolecular substances or gases, which are then repelled out. Pectinase, mannase, xylanase, and ligninase are key degumming enzymes (Zheng and Liu, 2004; Liu and Sun, 2018; Wang, 2009). Similar to PW, all superior degumming strains can secrete high level of pectinase, mannase, and xylanase, especially pectinase (Shu et al., 2020; Basu et al., 2009; Cheng et al., 2020).

Monosaccharide contents in fermentation broth

In the supernatant of kenaf bio-degumming fermentation broth, the detected hydrolysates included mannose, rhamnose, galacturonic acid, glucose, galactose, and xylose, while glucuronic acid was not detected. The liquid chromatogram is shown in Figure 3, and the concentrations of monosaccharide components in the fermentation broth are presented in Table 6. As the fermentation time progressed, the content of galacturonic acid showed a sustainable and slow growth, while the contents of other monosaccharides were first increased and then reduced. Among them, the contents of mannose, xylose, rhamnose, glucose, and galactose all reached the maximum values at 9 h.

The initial concentration of glucose was high, which might be correlated with the glucose components contained in the culture medium. The content of galacturonic acid, which was the pectin degradation product, was low and continuously increased. The reason might be that pectinase was a key enzyme with timely and radical microbial degradation and great demand. Its residual content was also not high in the supernatant of fermentation broth. During the degumming process, the kenaf hydrolysis products included mannose, rhamnose, galacturonic acid, glucose, galactose, and xylose. As the fermentation time was extended, the content of galacturonic acid presented a sustainable and slow growth trend, while the contents of other monosaccharides were first increased and then reduced. This finding proved that *Pectobacterium wasabiae* PW continuously released pectinase, mannase, and xylanase in the kenaf bast degumming process.

Note: 1. Mannose, 2. Rhamnose, 3. Glucuronic acid, 4. Galacturonic acid, 5. Glucose, 6. Galactose, 7. Xylose

Table 6 Concentration of monosaccharide component in the fermentation liquid from 0 h to 15 h (g/mL)

	Mannose	Rhamnose	Glucuronic acid	Galacturonic acid	Glucose	Galactose	Xylose
0 h	0.32	0	0	0.11	39.12	0	6.34
3 h	7.42	5.62	0	0.19	52.47	14.78	14.52
6 h	15.41	11.57	0	0.29	65.24	22.41	62.48
9 h	17.11	25.43	0	0.66	67.14	32.14	68.92
12 h	15.43	20.12	0	0.84	51.89	27.76	67.08
15 h	11.27	20.03	0	0.89	40.19	25.43	65.27

Degumming micro-detection

The kenaf fibers generated by bio-degumming and chemical degumming were observed under 3D video microscope by 100-fold amplification. As observed, the microfibers on the cellular wall of bio-degumming generated kenaf fiber were intersected and warped,

while those generated by chemical degumming were nearly under equal arrangement. The enzymes secreted by microorganisms selectively degraded colloids and reserved the inherent fiber morphologies and structures. In the chemical degumming process, strong alkali destructed the chemical and hydrogen bonds with weak structural force while hydrolyzing the colloids. Thus, the fibrous structure tended to be stable, and the excessive degradation decomposed bundle fibers into short single fibers. As a result, the mass of kenaf bundle fibers was reduced. The observation results under the electron microscope showed that, after 4 h degumming, the microorganisms infected the colloids by a large area and local degradation occurred. After 10 h, the single fibers were under obvious discrete state, the fiber surface was smooth, and most colloids already peeled cellulose off (Figure 4).

Comparison of kenaf degumming effects

The residual gum content of kenaf bast, fiber strength, and COD in the fermentation broth after oscillating fermentation of PW under optimized conditions for 12 h are shown in Table 7. The kenaf sample experiencing 15-day water retting degumming was collected for the control.

Table 7 Degumming effect in different degumming methods

	Residual gum content (%)	Weight loss ratio of raw jute (%)	Fiber strength (N)	COD (mg/L)
Bio-degumming	12.76	29.24	355	3045
Water retting	11.38	30.62	276	3582

Under bath ratio of 1:10, temperature of 33 ℃, and inoculum size of 2%, the bast fiber crops were washed after 12 h PW degumming. The residual gum content was 11.38%, which was 12.13% higher than that in the traditional water retting degumming. The raw material weight loss ratio was 4.51% lower than that in the traditional water retting degumming. However, the fiber strength in bio-degumming was 28.62% higher than that in traditional water retting degumming, and the COD in bio-degumming was 15.0% lower than that in traditional water retting degumming. The pectin removal rate of bio-degumming was 31.11% higher than that of water retting degumming, but the hemicellulose and lignin removal rates were 21.43% and 3.24% lower than those of traditional water retting degumming, respectively (Table 8).

Table 8 Chemical constituents of kenaf in different degumming methods

	Water soluble matter	pectin	hemicellulose	lignin	cellulose
Bio-degumming	0.8	0.93	11.56	10.2	76.51
Water retting	0.57	1.35	9.52	9.88	78.68

Wide spectrality of PW degumming function

PW is of good wide spectrality in the aspect of bast fiber crop degumming. After the fermentation for 12 h, the residual gum contents of kenaf bast, ramie bast, hemp bast, flax bast, and *Apocynum venetum* bast were all lower than 15%, and the raw material weight loss rate was 28.54%–34.70%. The residual gum content of *Apocynum venetum* was the minimum (12.57%) and that of flax was the maximum (15.07%). PW could complete the degumming of ramie bast, kenaf bast, hemp bast, flax bast, and *Apocynum venetum* bast. Therefore, it had excellent degumming wide spectrality. The fiber counts were greatly different. Specifically, those of kenaf and *Apocynum venetum* were 272 and 1,002 m/g, respectively. The COD ranged from 2,945 mg/L to 3,582 mg/L. In particular, the COD of ramie was the highest and that of kenaf was the lowest. In the traditional chemical soda cooking degumming process, the COD in boiling wastewater reached as high as 10,000 mg/L (research progress of wastewater treatment technology of ramie chemical degumming), which was much higher than that of bio-degumming wastewater. Among the current reported degumming strains, few can simultaneously realize the degumming of ramie, kenaf, hemp, and flax, but PW has favorable wide spectrality (Table 9).

Table 9 Degumming effect in different bast fiber crop materials

	Residual gum rate (%)	weight loss rate of raw material (%)	number of fibers (m/g)	COD (mg/L)
Kenaf bast	12.76	29.24	272	3045
Ramie bast	14.60	34.70	815	3582
Hemp bast	13.21	31.59	965	3267
Flax bast	14.89	28.54	927	3574
Apocynum venetum bast	12.57	30.05	1002	3119

Conclusions

A degumming bacterial strain *Pectobacterium wasabiae* PW with broad-spectrum degumming abilities was screened out in this study. After the fermentation for 12 h, the residual gum contents were lower than 15%. This bacterial strain could realize the synchronous extracellular secretion of pectinase, mannase, and xylanase with the maximum activity levels of 130.25, 157.58, and 115.24 IU/mL, respectively. The optimal degumming conditions of this bacterial strain were as follows: time of 12 h, bath ratio of 1:10, temperature of 33 °C, and inoculum size of 2%. The COD in bio-degumming wastewater was below 4,000 mg/L, which was over 60% lower than that in boiling wastewater of chemical degumming.

Declarations

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Authors' contributions Shengwen Duan: Methodology, Investigation, Writing - original draft. Bingrong Xu: Methodology, Investigation, Writing - original draft. Lifeng Cheng: Supervision, Conceptualization, Writing - review & editing. Xiangyuan Feng: Supervision, Conceptualization, Writing - review & editing. Qi Yang: Formal analysis, Software, Validation. Ke Zheng: Formal analysis, Software, Validation. Zewei Ma: Software. Mingqiang Gao: Formal analysis, Software, Validation. Yuande Peng: Supervision, Conceptualization, Writing - review & editing.

Availability of data and materials The strain *Pectobacterium wasabiae* PW was preserved in China General Microbiological Culture Collection Center (CGMCC), with the preservation number of CGMCC No. 14601.

Code availability Not applicable.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with the work presented.

Ethics approval Not applicable.

Human and animal rights participants Not applicable.

Informed consent Not applicable.

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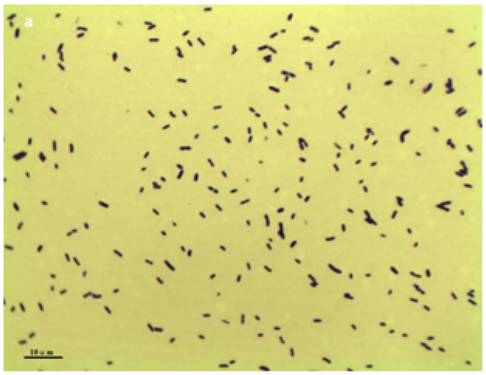
References

1. Biswas D, Chakrabarti SK, De S, Paral R (2016) Eco-friendly degumming technology for ramie fiber. J Nat Fibers 132:227–237

2. Crini G, Lichtfouse E (2020) Sustainable agriculture reviews 42, hemp production and applications. Cham, Switzerland; Springer
3. Cheng LF, Duan SW, Feng XY, Zheng K, Yang Q, Xu H, Luo W, Peng YD (2020) Screening and identification of pectinolytic bacteria for ramie degumming. Text Res J DOI. 10.1177/0040517520968280
4. Deng YM (2010) Natural textile fiber processing chemistry. Southwest Normal University Press
5. Ding RY, Zhang XQ, Yu CW (2014) Optimization of enzyme mixture degumming of ramie fiber. J Nat Fibers 11:13–24
6. Do VH, Tran PL, Ni L, Park KH (2016) A continuous coupled spectrophotometric assay for debranching enzyme activity using reducing end-specific α-glucosidase. Anal Biochem 492:21–26
7. Duan SW, Liu ZC, Feng XY, Zheng K, Cheng LF, Zheng X (2012) Diversity and characterization of ramie-degumming strains. Sci Agr 69:119–125
8. Duan SW, Feng XY, Cheng LF, Peng YD, Zheng K, Liu ZC (2016) Bio-degumming technology of jute bast by Pectobacterium sp. DCE-01. AMB Express 6:86
9. Duan SW, Cheng LF, Peng YD, Feng XY, Zheng K, Yang Q, Liu ZY (2018) Enzyme preparation for bast degumming and its degumming process. China invention patents, ZL(2017)10942753
10. Duan SW, Cheng LF, Liu ZC, Feng XY, Zheng K, Peng YD (2018) Diversity and characteristics of kenaf Bast degumming microbial resources. J Nat Fibers 15:799–807
11. Fan P, He F, Yang Y, Ao MZ, Ouyang J, Liu Y, Yu LJ (2015) In-situ microbial degumming technology with Bacillus sp. HG-28 for industrial production of ramie fibers. Biochem Eng J 97:50–58
12. Fang JJ, Qin GC, Ma J, She YM (2015) Quantification of plant cell wall monosaccharides by reversed-phase liquid chromatography with 2-aminobenzamide pre-column derivatization and a non-toxic reducing reagent 2-picoline borane. J Chromatogr A 1414:122–128
13. Jiang FC, Shao K (2005) Method of quantitative analysis of ramie chemical components GB 5889 – 1986. Standards Press of China, Beijing (in Chinese)
14. Liu ZC (2009) Progress on the Science and Technology of Bio-extrac -tion of Bast Fibers. Plant Fiber Sciences in China 31:93–97
15. Liu ZC (2013) Progress on applicaton of biological agents in process- ing industry for agricultural products of herbaceous fiber. Journal of Agricultural Science Technolog 5:17–23
16. Liu ZC, Sun QX (2018) Science and engineering of herbaceous fiber biorefinery. China Agricultural Science and Technology Press
17. Pei F (2015) In-situ microbial degumming technology for ramie fiber production: research and development.Wuhan. Huazhong University of Science and Technology
18. Shao K (2003) Textile processing and chemical. China Textile Press, Beijing
19. Shu T, Bai Y, Wang YW, Wang HH, Li PD, Xiang MX, Yu TY, Xu H, Yu LJ (2020) A high-efficiency and eco-friendly degumming process for ramie fibers. J Clean Prod 276:124217
20. Snehasish B, Manabendra N, Saha DC, Krishanu C (2009) Large-s cale degumming of ramie fibre using a newly isolated Bacillus pumilus DKS1 with high pectate lyase activity. J Ind Microbiol Biot 36:239–245
21. Subasinghe ADL, Das R, Bhattacharyya D (2016) Parametric analys- is of flammability performance of polypropylene/kenaf composites. J Ma -ter Sci 51:2101–2111
22. Swarupa R, Chiliveri SK, Venkateswar RL (2016) Retting and degumming of natural fibers by pectinolytic enzymes produced from bacillus tequilensis sv11-uv37 using solid state fermentation. SpringerPlus 5:559
23. Wang XS (2009) Gene clong and expression of enzymes for bio-extrac- ting of herbaceous fiber Chinese academy of agricultural sciences. Chinese Academy of Agricultural Sciences, Beijing
24. Wang YW, Shu T, Fan P, Zhang HS, Ossi T, Xiong HR, Yu LJ (2017) Characterization of a recombinant alkaline thermostable β-m-annanase and its application in eco-friendly ramie degumming. Process Biochem 61:73–79
25. Xiong HP (2008) Bast fiber crops breeding. China Agricultural Science and Technology Press
26. Yang YZ (2016) The study on the functional effect of acetyl xylan esterase in ramie bast fiber microbial degummingprocess. Wuhan, Huazhong University of Science and Technology
27. Yang Q, Duan SW, Peng YD (2018) Research Development on Microbial Degumming of Ramie. Plant Fiber Sciences in China 40:36–42
28. Zhang JC (2005) Hemp comprehensive utilization technology. Great Wall press

29. Zhang SJ, Li CL, Zhou GY, Che GD, You JM, Suo YR (2013) Determination of the carbohydrates from Notopterygium forbesii Boiss by HPLC with fluorescence detection. Carbohydr Polym 97:794–799
30. Zheng LS, Du YM, Zhang JY (2001) Degumming of ramie fibers by alkalophilic bacteria and their polysaccharide-degrading enzymes. Bioresource Technol 78:89–94
31. Zheng LJ (2007) A study on the degumming and modification of kenaf bast fiber by bio-enzyme degradation. Shanghai, Donghua Univer- sity
32. Zheng LJ, Liu JY (2004) Influencing factors and mechanism of microbial degumming of Jute/Kenaf. Journal of Donghua University (Natural Science) 3:66–70
33. Zhou C, Xue YF, Ma YH (2017) Characterization and over producti- on of a thermo-alkaline pectate lyase from alkaliphilic Bacillus liche -niformis with potential in ramie degumming. Process Biochem 54:49–58
34. Zhou C, Ye J, Xue Y (2015) Directed evolution and structural analysis of alkaline pectate lyase from the alkaliphilic bacterium Bacillus sp. strain N16-5 to improve its thermostability for efficient ramie degumming. Appl Environ Micro 8117:5714–5723

Figures



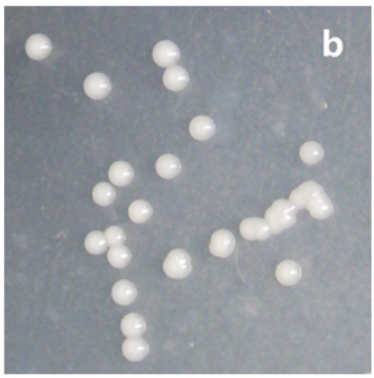


Figure 1

(a) Microscopic morphology, (b) colonial morphology of Pectobacterium wasabiae strain PW

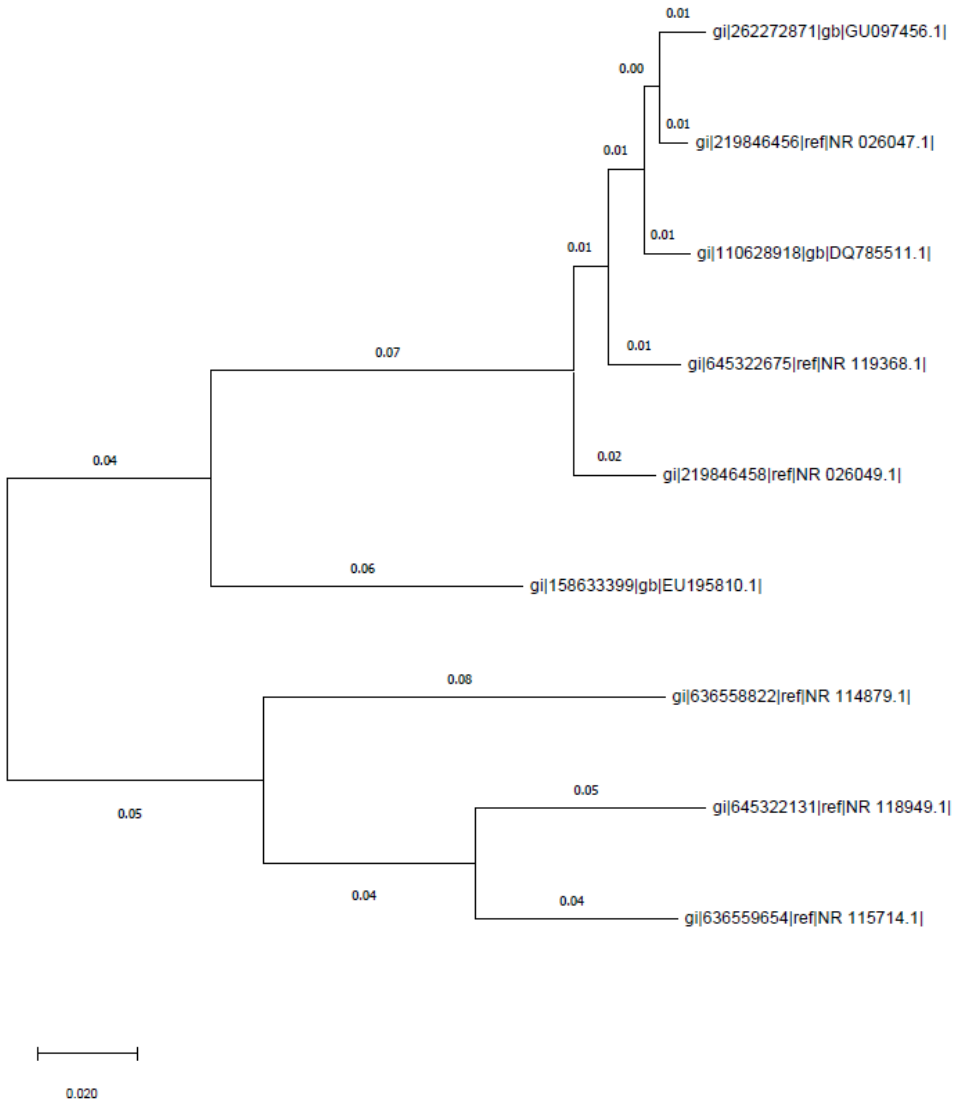


Figure 2

Phylogenetic tree generated using the minimum-evolution method based on the 16S rRNA gene sequence of strain PW. Numbers at nodes represent bootstrap percentages based on 1,000 samplings. Bar, 0.02 changes per nucleotide position. The neighbor-joining tree showed the same topology (data not shown)

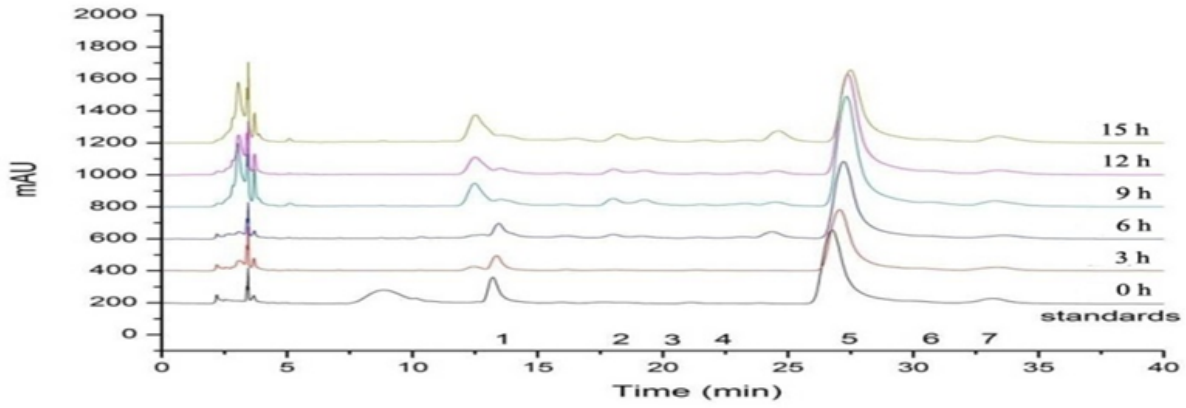
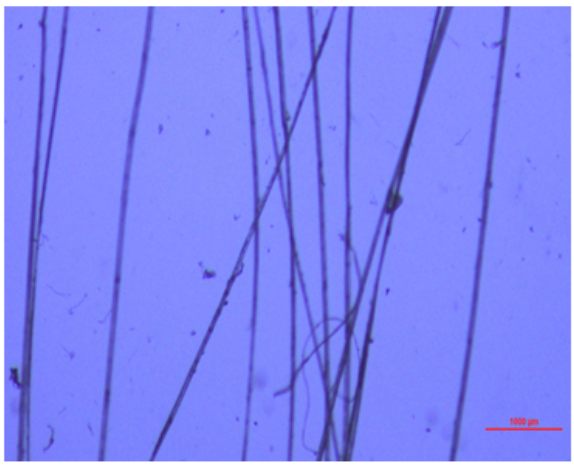
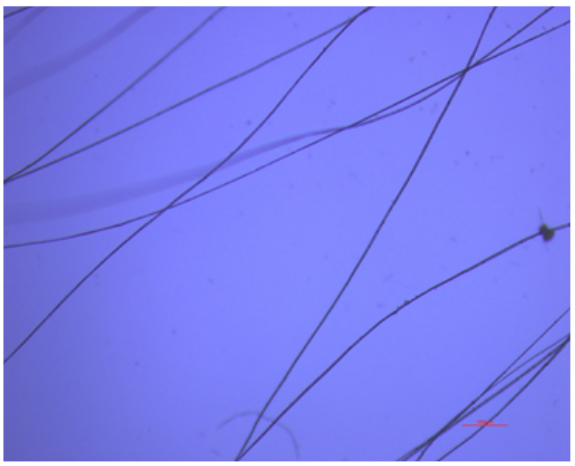


Figure 3

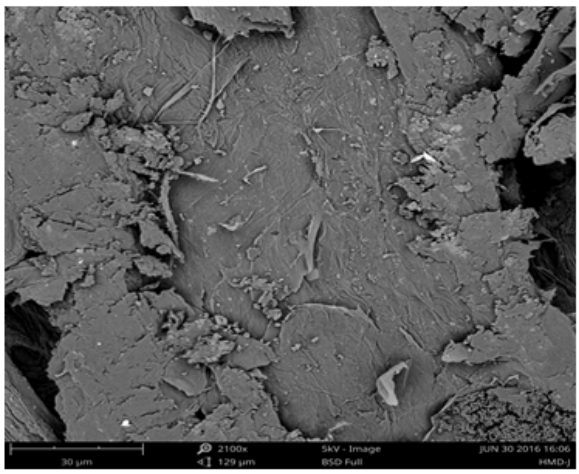
Monosaccharide chromatogram of kenaf fermentation broth



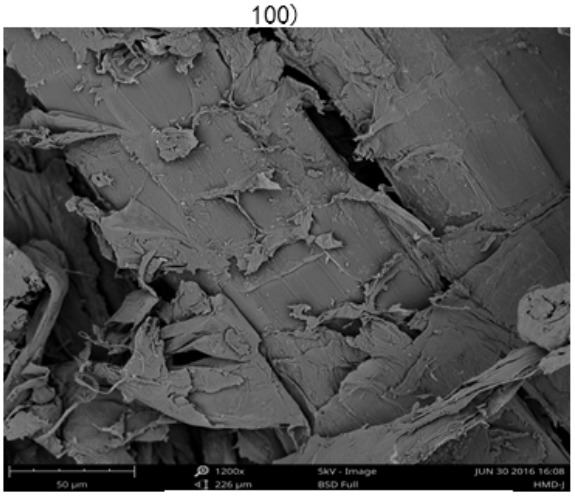
Bio-degumming (×100)



Chemical degumming (×100)



4 h microbial infection and enzymolysis



Single fiber was formed after 10 h catalytic degradation

Figure 4

Microscopic detection during degumming process