The isolation and functional identification on producing cellulase of Pseudomonas mendocina

Jianfeng Zhang, Hongyan Hou, Guang Chen, Shusheng Wang, and Jiejing Zhang

Bioengineering Department, School of Life Sciences, Jilin Agricultural University, Jilin Province Innovation Platform of Straw Comprehensive Utilization Technology, Changchun, P. R. China

ABSTRACT

The straw can be degraded efficiently into humus by powerful enzymes from microorganisms, resulting in the accelerated circulation of N, P, K and other effective elements in ecological system. We isolated a strain through screening the straw degradation strains from natural humic straw in the low temperature area in northeast of China, which can produce cellulase efficiently. The strain was identified as Pseudomonas mendocina by using morphological, physiological, biochemical test, and molecular biological test, with the functional clarification on producing cellulase for Pseudomonas mendocina for the first time. The enzyme force constant Km and the maximum reaction rate (Vmax) of the strain were 0.3261 g/L and 0.1525 mg/(min.L) through the enzyme activity detection, and the molecular weight of the enzyme produced by the strain were 42.4 kD and 20.4 kD based on SDS-PAGE. The effects of various ecological factors such as temperature, pH and nematodes on the enzyme produced by the strain in the micro ecosystem in plant roots were evaluated. The result showed that the optimum temperature was 28°C, and the best pH was 7.4–7.8, the impact heavy metal was Pb^{2+} and the enzyme activity and biomass of Pseudomonas mendocina increased the movement and predation of nematodes.

KEYWORDS

Cellulase producing strain; characterization of enzyme; nematodes; Pseudomonas mendocina; strain identification

Introduction

As the main organic matter in nature, Celllose accounts for 1/3 of annual plants and accounts for 1/2 of perennial plants, which plays an important role in the carbon cycle. The complex fraction of cellulose makes it difficult to degrade, which is one of the major pollution sources of air, water and soil from agriculture. Cellulose molecules with a complex structure of crystalline molecular are formed by adjacent D-glucose residues with β-1,4-glycosidic bonds. Straw is the main agriculture pollution source, and the primary component is cellulose. At present, the straw processing methods include chemical, physical and biological methods.

Cellulase is a multicomponent enzyme system, and can be mainly divided into 3 categories according to the different function (Fig. 1): Endoglucanase (CX), Exoglucanases (CI) and Cellobiase (BG). CX effect on the amorphous regions inside the cellulose molecules and hydrolyze the β-1,4-glycosidic bonds randomly, so it divides the long chain cellulose molecules into a large number of small cellulose molecules with nonreducing end. CI hydrolyzes the linear cellulose molecules into Cellobiase, Cellobiase is hydrolyzed into glucose by BG finally. A large variety of microbes can produce cellulase. White rot fungus can destruct the straw surface waxy layer and dense structure, damage the cell wall massively and degrade the straw from the internal organization.

As a main soil animal, Nematodes are widely distributed in various habitats as the function soil animal species, which play an important role in soil ecosystem. The response of soil microbes to nematodes is mainly reflected in the changes of biomass, microbial activity and microbial community structure. The researchers found that nematodes could change the genetic structure of microbial communities with denaturing gradient gel electrophoresis (DGGG) through investigating 4 kinds of bacterivorous nematodes using microbial community culture system.

The aim of this paper is to investigate the interaction of various factors in micro ecosystem on the straw.
degradation strains. The wild strain with the efficiency of straw degradation was selected from rotted straw in the low temperature area in northeast of China. We selected strains by the means of Congo staining, cellulase activity and characters. Then we identified one strain, which could produce high active cellulase. We identified the strain by morphological, physiological and biochemical techniques, and the strain was identified as Pseudomonas mendocina (PME). In the study of Lednicka found that PME could degrade fiber, but it did not research the character of PME until now. This paper studied the type of enzyme, and the paper probes on how heavy metal, temperature, pH and other micro animal effects on enzyme production.

Materials and methods

The samples with pH 7.2 were collected from rotten straw in Changchun city, Jilin province in northeast area of China (43°05′ to 45°15′ N and 124°18′ to 127°05′E) in 2011, and collected at −4 °C until cellulase strain screened. The strain was identified as Pseudomonas mendocina through physiological biochemical test and 16 SrDNA. The strain was sent to China General Microbiological Culture Collection Centen (CGMCC). The accession number is CGMCC No.6711. Furthermore, Nematode was provided by the Lab of Soil microbial diversity, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences.

Enrichment medium (Table 1): K₂HPO₄ 2 g, (NH₄)₂SO₄ 1.4 g, MgSO₄•7H₂O 0.3 g, CaCl₂ 0.3 g, FeSO₄•7H₂O 5 mg, MnSO₄ 1.6 mg, ZnSO₄ 1.7 mg, CoCl₂ 2 mg, Xinhua 1 filter paper 2 g, pH 5.5, 121 °C sterilize 30 min. Screening culture medium: CMC 10 g/L, (NH₄)₂SO₄ 4 g/L, KH₂PO₄ 2 g/L, MgSO₄•7H₂O 0. Five g/L, Peptone 1 g/L, Agar 18 g/L, pH 6.5–7.0. Liquid fermentation medium: CMC-Na 0.5 %, Peptone 0.3 %, (NH₄)₂SO₄ 0.2 %, KH₂PO₄ 0.4 %, CaCl₂ 0.03 %, MgSO₄•7H₂O 0.03 %, Tween-80 0.02 %, Nature pH, 121 °C sterilize 20 min.

The collected samples were diluted into suspension of 10⁻³–10⁻⁸. 1 mL dilution was plated into enrichment medium and cultured for 72 h at 30 °C, repeated every dilution for 2 times. The strain was selected with maximum EL and fermented in the fermentation culture medium. The fermentation was detected CMCase activity and filter paper activity (FPA) respectively.

Figure 1. The influence mechanism of external ecological factors on humic substances degradation by microbial. The cellulase genes were expressed in the induction of plant residues and produced CX, CI, CB 3 kinds of extracellular enzymes, which degraded the cellulose in the plant into glucose, moreover produced available N, P, K, S and other substances during the degradation proceeding. Nematodes and other ecology factors can affect the plant residues degradation.
Subsequently, cellulase properties of dynamic constant Km and molecular weight were detected. To identify the strain, the methods of morphological, physiological and biochemical, molecular biological techniques were applied. The effect of temperature on the cellulase activity: Determined the optimum temperature and stability of the enzyme reaction per 4 °C in the range of 20 °C ~60 °C.17 The effect of pH on the cellulase activity: Determined the optimum pH and stability of the enzyme reaction per 0.5 grad in the range of pH 5~8.18

The effect of heavy metal on the cellulase activity: Detected the promotion or inhibition of enzyme reaction added heavy metal with K+, Ca2+, Cu2+, Fe3+, Mg2+, Pb2+, Zn2+ and Mn2+.19 The effect of nematodes on the cellulase activity: The seed liquid of the strain was diluted to 2 × ~5 × and inserted into fermentation medium and solid medium, which inserted nematodes with the concentration of 90/100 μL, then detected the predation and movement of the nematodes and the enzyme reaction of the strain.15,20 Diluted the metabolites of nematodes to the gradient of 1×~3×, which cultured 6 h with the concentration of 90/100 μL, and inserted to the fermentation solution with strain, then determined the effect of nematodes on enzyme activity of the strain.

The suspension culture mass in the screening culture medium was picked up, the medium with Congo was strained and purified several times and screened out the strain with maximum enzymatic reaction index EI.15 EI = d1/d2. d1:dimater of hydrolysis zone; d2:dimer of colony.

Extracted enzyme solution was detected for CMCase activity and filter paper activity (FPA) respectively. Added the enzyme solution 0.5 mL and 1% CMC-Na buffer into tube to heat 30 min at 50 °C, and determined CMCase; Added enzyme solution 0.5 mL and 1 mL citric acid buffer to heat 1 h at 50 °C, and determined FPA;21 Then, determined reducing sugar with the method of DNS and represent the enzyme activity with international units 1 μmol/min. mL.22 Choose the strain with highest cellulase activity and determined the filter paper disintegration and straw degradation tests in the flask, so as to observe the degradation ability.

Determination of enzyme dynamic constant Km: Allocated the concentration of CMC-Na buffer to 0.75 g/L, 1.25 g/L, 1.75 g/L, 2.00 g/L, 2.25 g/L, 2.50 g/L, and determined the cellulase activity using DNS method at pH6.0, 50 °C, calculated the initial reaction velocity of the enzyme.23 Made the double reciprocal plot of Line-weaver-Burk, the abscissa of which is the reciprocal of substrate concentration (1/S) and the ordinate is the initial velocity of enzymatic reaction (1/V), then received the line and calculated Vmax and Km: 1/V = Km/ Vmax×1/[S]+1/Vmax.24 1/Vmax: intercept of ordinate axis, K/Vmax: slope, 1/Km: intercept of absissa axis.

SDS-PAGE: Used the method of discontinuous vertical plate polyacrylamide gel electrophoresis referring to Lemlli.25 Configured 12.5 % separating gel and 4 % stacking gel, and the thickness of which is 1.5 mm. 5 μL sample was dipped in the gel, and the voltage was adjusted to 80 v, then adjusted the voltage to 100 v when the sample into the separating gel, and stopped electrophoresis when the indicator arrived at 2~3 cm from rubber. Strained the gel 1 h using Using Kaumas’s brilliant blue R-250, rinsed 2~3 times with distilled water, and decolorized on the decolorization Shaker with bleaching liquid (ethanol: acetic acid: water = 4:1:5) until each sub-unit on the gel clearly.20 The calculation of results: ① Calculation of the relative migration rate: measured the distance of sample zone center and indicator area center, and calculated the relative migration with the formula as fellow: Relative migration = L1/L2. L1: the distance of protein movement(cm); L2: the distance of indicator movement(cm). ② Draw the curve of the protein standard and determined the relative molecular weight of the unknown protein: Made the diagram of Rf-LogmR, the absissa of which was relative migration rate of the protein and the ordinate was the relative molecular mass logarithmic of the correspond protein, and calculated the molecular weight of cellulase.

Morphological identification of the strain: Identified the strain from the morphology of the growth

---

**Table 1. Reagents list.**

| Reagent       | Company                                      | Catalog number        |
|---------------|----------------------------------------------|-----------------------|
| EDTA          | Shanghai Chemical Reagent Co. Ltd.           | F20120607             |
| CTAB          | Sigma                                        | 575105                |
| SDS           | Ruibo                                        | BME169                |
| EB            | Beijing Labest Biological Technology Co. Ltd. | Amresco 0492         |
| K2HPO4        | Tianjin Zhiyuan Chemical Reagent Co., Ltd.   | 20110004169           |
| (NH4)2SO4     | Beijing Chemical Works                       | 201201030             |
| MgSO4·7H2O    | Shanghai national drug group                 | 20110811              |
| CaCl2         | Tianjin Zhiyuan Chemical Reagent Co., Ltd.   | 20110302              |
| FeSO4·7H2O    | West Long Chemical Factory                   | 1016012-01-09         |
| MnSO4         | Shanghai national drug group                 | 20091120              |
| ZnSO4         | Tianjin Bodi Chemical                        | 20100327              |
| CoCl2         | Tianjin Bodi Chemical                        | 20111107              |
| Agar          | Japan A 7064                                 | QA7064                |
| CMC-Na        | Tianjin Zhiyuan Chemical Reagent Co., Ltd.   | 20110815              |
| Congo         | Shanghai Chemical Reagent Co., Ltd.          | 20091120              |
state, color, shape, surface, roughness, gloss, transparency, edge shape and growth status, which growth on the plate medium.\textsuperscript{26}

Physiological and biochemical identification: Identified the strain from the physiological and biochemical tests of Gram staining, cell shape, catalase test, starch hydrolysis test, H\textsubscript{2}S test, V-P test, MR test, anaerobic growth, enzyme reaction, glucose oxidation fermentation, high salt tolerance, Denitrification, malonate utilization, gelatin liquefaction, Arg dihydrolase, citrate utilization, glucose, xylose, xylobol, lactose, indole, Arabinose, trehalose and sucrose test, which accorded to Bergey’s Manual of Determinative Bacteriology.\textsuperscript{6}

Molecular biology identification: Genomic NDA was picked up the by CTAB method and amplified by PCR with universal primer 1 and primer 2 of bacterial.\textsuperscript{27} Primer 1: 5’-GCCTAACACATGCAAGTCGA-3’, Primer 2: 5’-GTATTACCGCGGCTGCTGG-3’. The primers were composed by Beijing Huada Biological Engineering Technology Co., Ltd. The sequence of the PCR products were determined and sequenced by BLAST.

We determined the cellulase activity of the strain fermentation with different temperature, pH and heavy metals. We also determined the predation rate of nematode in the proceeding of effect on enzyme activity according the following formula: Nematode predation rate = (nematode amount in experimental group + nematode egg in experimental group - nematode egg after cultured)/(nematode amount in control group + nematode egg in control group) \times 100 \%.\textsuperscript{28}

Results

CMC-Na and CMC could be dyed into red combined with Congo, and the color would be lighter while they were degradation into reducing sugar,\textsuperscript{29} then 10 strains were screened out with different shapes and

\begin{center}
\includegraphics[width=\textwidth]{figure2.png}
\end{center}

\textbf{Figure 2.} Morphological indicator of the strain. (A) The pictures of hydrolysis circle of the N\textsuperscript{-1} strain; (B) Morphological of N\textsuperscript{-1} strain on the medium; (C) Gram staining morphological of N\textsuperscript{-1} strain.

\begin{center}
\includegraphics[width=\textwidth]{figure3.png}
\end{center}

\textbf{Figure 3.} The Lineweaver-Burk, the reciprocal of substrate concentration (I/S) and the ordinate is the initial velocity of enzymatic reaction (I/V), then acquired formulas as fellows: \( y = 2.1388x + 6.4964 \), \( 1/V_{\text{max}} = 6.4964 \), \( Km/V_{\text{max}} = 2.1388 \), and calculated Km and Vmax.

\begin{center}
\includegraphics[width=\textwidth]{figure4.png}
\end{center}

\textbf{Figure 4.} The SDS-PAGE electrophoresis of the sample and standard protein Marker. 1: Marker; 2: The sample of extracellular enzymes of the strain in the fluid.
transparent circle and purified the strains and collected the maximum one (Fig. 2A). The screened strain was named N”-1. In order to verify the hydrolysis circle of maximum strain with maximal enzyme activities, we extracted the crude enzyme solution and determined the CMCase and FPA, by which we found that N”-1 had the highest enzyme activity. The CMCase was 92 IU and the FPA reached 97 IU. The filter paper disintegrating degree and straw degradation degree reached the fourth level +++++, and presented mushy and rotten shape respectively.

The double reciprocal plot of Lineweaver-Burk was made, the abscissa of which is the reciprocal of substrate concentration (l/S) and the ordinate is the initial velocity of enzymatic reaction (l/V) (Fig. 3). Calculated the intercept X and Y axis intercept according to the linear regression equation \( y = 2.1388x + 6.4964 \). The ordinate intercept was 1/Vmax ; the abcissa intercept was 1/Km and the slop was Km/Vmax, then got the Km as 0.3261 g/L and the Vmax of enzyme power as 0.1525 mg/ (min.L) for CMC-Na in pH 6.0 at 50 °C.

Eccentric the bacterial fermentation liquid under 6000 r/min for 20 min and extracted the crude enzyme solution, then picked up the supernatant of the crude enzyme solution after centrifugal under 12000 r/min for 1 min as sample, and electrophoresis by SDS-PAGE with standard protein marker (Fig. 4). Measured the distance of sample zone center and indicator area center, and calculated the relative migration (Rm) and calculated the relative molecular mass logarithmic (Lg(MW)) of the standard protein (Table 2).

Made the standard curve according to Lg (MW) and Rm (Fig. 5), so that we can draw the cellulase molecular weight of extracellular fluid. The Rm of the sample was 0.337 and 0.604, and cellulase molecular weights were 42.4 kDa and 20.4 kDa, which calculated according to the standard curve.

The result of the strain morphology identification: The strain was cultured into a single colony in the medium and observed the morphology. The characterization of the strain as follows: the color was white, the surface rendering state was half moist, the single colony has a large flattened shape, and the edge was serrated and rapid growthly (Fig. 2B). At the same time, we detected the Gram straining of the strain and found which was negative and rod (Fig. 2C). Therefore the strain was preliminary identified as bacteria through the morphology identification.

The result of the strain physiological and biochemical identification: Determined the physiological and biochemical character of the strain according to Bergey’s Manual of Determinative Bacteriology (Table 3) and was identified as Pseudomonas spp.

The result of the molecular biology identification: Extract the DNA from the strain fermentation fluid and amplify by the means of PCR with the bacterial universal primers of Primer 1 and Primer 2. Take 2 μL products of PCR and electrophoresed in agarose gel with the Marker of 2000 bp, and receive the electrophoresis results (Fig. 6A) which found that the amplificatory fragment was approximately 500 bp. Measure the sequence of PCR products and compared with BLAST in NCBI. The full length of N”-1 sequence was 525 bp, which was submitted in Genbank and obtained the accession number Id154117. Two sequences of the gene were considered as homology when they have more than 98 % similarity. We found that the strain of N”-1 was highly similar with Pseudomonas mendocina (PME) (Fig. 6B).

The enzyme activity and biomass of PME changed under different physical ecological factors of pH, temperature and heavy metal, as well as the result of those changes were shown in Fig. 7. The optimum pH was 7.4~7.8 for the strains’ growth and enzyme producing (Fig. 7A), which showed that the CMCase, FPA and biomass reached ultimate value during pH 7.4 and 7.8.

| Project          | Standard protein |
|------------------|------------------|
| Relative migration (Rm) | 0.069 0.165 0.395 0.521 0.705 |
| Molecular weight (Kd)  | 57.2 66.4 29.0 20.1 14.3 |
| Lg (MW)            | 1.987 1.822 1.462 1.303 1.155 |
At the same time, the study found that PME could adjust the metabolism function in a range of pH. PME has a wide temperature range, while the enzyme activity began to rise stably when the temperature up to 28 °C (Fig. 7B). Pb^{2+} was the capital heavy metal for PME in the process of enzyme producing, but it would enhance the biomass and enzyme activity of PME when the heavy metal was added adequately such as K^+, Mg^{2+}, Fe^{3+} (Fig. 7C).

In the study of cellulase activity influenced by nematode found that the transparent circle of PME cultured in the medium added with nematodes is obviously higher than which without nematodes, and nematodes could increase the CMC degradation efficiency of PME during the proceeding of predation and movement (Fig. 8A). The enzyme activity of the control group and experimental group reached maximum value when they fermented for 32 h. The enzyme activity in experimental group injected with PME arrived 101 IU, while the control group without nematodes only 92 IU, and the raised by 10 % (Fig. 8B).

The effect of PME on nematodes predation rate was shown in Fig. 9A. The result showed that the predation rate of nematodes was rising in the first 24 h, but began to die after that and the death rate was 100 % at the 72 h. The effect of nematode metabolites on the cellulase activity of PME was shown in Fig. 9B. Compared with the control group, the enzyme activity in the experimental group were highly increased and the higher concentrations of enzyme the greater of the activity.

**Discussions**

As an important agricultural management technique, straw degradation not only increases the organic carbon content in soil but also provides nutrition for plants and microorganism. The carbon level is the important indicator of soil laborious rising, as well as a way of mitigating global warming by soil carbon sequestration. Therefore, there are vital production and ecological benefit to degrade straw. It can provide a large number of high quality organic fertilizer for agriculture by straw decomposition using microorganism, It is not only beneficial to the economy, environment and society, but also provides a reasonable way of reusing the straw.

It has been well known that cellulase can degrade cellulose specifically and the activity may differ in the different substrates, which means different substrate can induce cellulase with different enzyme activity. We want to screen the natural strain, which can degrade straw in the rooted straw in the Jilin province of China in this study. Induced by the plant residual, the express of cellulase genes can
produce cellulase, and then we used the screening culture medium with the sole carbon source of CMC-Na and dyed the medium with Congo, and screened the strains with large transparent circle, then determined the enzyme activity of the strains (Fig. 2). During the proceeding of study on enzyme activity we found that compared with the previous study on the strain producing cellulase, the strain collected had higher enzyme activity. In the previous study, the highest enzyme activity of cellulose degradation for bacteria was 83.05 IU as GU,W et al and the enzyme activity of the collected strain was 97 IU.\(^3\) Therefore, the study needs to identify bacteria of the strain and identified as bacteria through morphological test (Fig. 2), was identified as *Pseudomonas* bacteria according to physiological and biochemical determination (Table 3). Finally, the strain was identified as *Pseudomonas mendocina* (PME) by molecular biology method of DNA fragment amplified and Blast in NCBI (Fig. 6).

PME was originally isolated from soil and water samples, which belongs to the environmental microbes and the uncoupling anaerobic microorganism.\(^3\) PME is a Gram-negative bacterium in the environment and could cause nosocomial infections in the clinical in the previous studies such as infective spondylodiscitis endocarditis.\(^3\) Besides its application in the clinical fields, PME has also the potential use to degrade toluene in bioremediation.\(^3\) PME is also mainly compose of sugar and lipid and oil degradation in the previous.\(^3\) PME has been placed the

![Figure 7](image_url)

**Figure 7.** The enzyme activity and biomass of PME changed under different physical ecological factors. (A) The influence of initial pH on enzyme activity of the strain; (B) The influence of temperature on enzyme activity of the strain; (C) The influence of heavy metal on enzyme activity of the strain.

![Figure 8](image_url)

**Figure 8.** (A) The effect of nematodes on CMC degradation of PME; (B) The changes of cellulase activity under nematodes (D: Control group without nematodes; NP: Experimental group with PME and nematodes).
P. Aeruginosa Nk-01 encodes produced PHAMCL and AO synthesis substrates based on 16 S rRNA. However, PME’s function of producing cellulase was never discovered in previous studies. This paper was the first report for recognizing the cellulase production function of PME. By studying strain characters we found that the enzyme force constant Km of PME was determined for the first time (Fig. 3) and figured out the Km as 0.3261 g/L and the Vmax of enzyme power as 0.1525 mg/(min.L) for CMC-Na in pH6.0 at 50°C. The molecular weight of the cellulase of PME was first examined. Through the SDS-PAGE analysis of protein standard achieved the standard curve (Fig. 5), the cellulase was figure out by measuring the relative mobility and the cellulase molecular weight were 42.4 kD and 20.4 kD.

The enzyme activity and biomass of PME changed under different physical ecological factors of pH, temperature and heavy metal (Fig. 7). Strong acid or alkali soil is not suitable for the growth of strains, and suitable pH can provide a suitable environment for the growth of bacteria and enzyme activity. The optimum pH was 7.4~7.8 for the growth and enzyme producing of PME, and could adjust the metabolism function in a range of pH. The suitable temperature has important function on enzyme activity, and PME has a wide temperature range. Heavy metals had initially polluted on soil, leading to sustainable growth of forests and crops, which had effected the proceeding of metabolism of the soil microbial and plant in the environment of microecology. Pb²⁺ was the capital heavy metal for PME in the process of enzyme producing. It could enhance the biomass and enzyme activity of PME when the heavy metal was added adequately such as K⁺, Mg²⁺, Fe³⁺.

There is an important ecological factor on the degradation of straw, nematodes play an important role in the microecology, which affected the cellulase activity of PME (Figs. 8 and 9). In the study of nematodes showed that the higher concentration of PME the higher of the cellulase activity and the role factor was PME, while nematodes were the auxiliary degradation of the fiber. There are 2 reasons: first, the metabolic products of PME with some components inhibit nematode growth, second the nematode should be raised with enough oxygen in the process of cultivation in the liquid culture. The movement and metabolite of the nematodes could accelerate the proliferation and enzyme activity of PME.

In summary, during the proceeding of cellulase producing strain we firstly found that Pseudomonas mendocina (PME) has the function of degrading CMC-Na, and the cellulase activity was higher than actinomyces reported in the former study. Then, the research determined the characteristics of the cellulase produced by PME. Result showed that the cellulase was extracellular enzyme, and kept high activity in the suitable external environment factor under the influence of the activity.

Acknowledgments
We are thankful for the help from Professor Chunjie Tian, Xiaomei Wang and Dr. Lixing Wei, Bingsheng Lv, Hongwei Geng.

Funding
The authors would like to thank the financial support from Changchun Administration of Science & Technology (Grant No.2013173), the education department of Jilin province (2016176) and Jilin Agricultural University (201409).

References
[1] Schädel C, Blöchl A, Richter A, Hoch G. Short-term dynamics of nonstructural carbohydrates and hemicelluloses in young branches of temperate forest trees during bud break. Tree Physiol 2009;29:901-11; http://dx.doi.org/10.1093/treephys/tpp034
[2] Li Y, Tian C, Tian H, Zhang J, He X, Ping W, Lei H. Improvement of bacterial cellulose production by manipulating the metabolic pathways in which ethanol and
sodium citrate involved. Appl Microbiol Biotechnol 2012;96:1479-87; PMID:22782249; http://dx.doi.org/10.1007/s00253-012-4242-6

[3] Sarah M, Ely M, Yoav B, Dan G, Yitzhak H, Raphael L, Yuval S, Wilson DB, Bayer EA. Deconstruction of lignocellulose into soluble sugars by native and designer cellulases. Mbio 2012;3:214104-4.

[4] Chiueh PT, Lee KC, Syu FS, Lo SL. Implications of biomass pretreatment to cost and carbon emissions: Case study of rice straw and Pennisetum in Taiwan. Bioresour Technol 2012;108:285-94; PMID:22281146; http://dx.doi.org/10.1016/j.biotechnol.2012.01.006

[5] Zhang YHP, Himmel ME, Mielenz JR. Outlook for cellulase production and selection strategies. Biotechnol Adv 2006;24:452-81; PMID:16690241; http://dx.doi.org/10.1016/j.biotechnol.2006.03.003

[6] Zhang L, Guo ZP, Ding ZY, Wang ZX, Shi GY. Construction of the industrial ethanol-producing strain of Saccharomyces cerevisiae able to ferment cellobiose and melibiose. Prikli Biokhim Mikrobiol 2012;48:243-8; PMID:22586919

[7] Ha SJ, Galazka JM, Kim SR, Choi JH, Yang XM, Seo JH, Glass NL, Cate JHD, Jin YS. Engineered Saccharomyces cerevisiae capable of simultaneous cellobiose and xylose fermentation. Proc Natl Acad Sci U S A 2011;108:5004-9; PMID:21187422; http://dx.doi.org/10.1073/pnas.1010456108

[8] Weiss N, Borjesson J, Pedersen LS, Meyer AS. Enzymatic lignocellulose hydrolysis: Improved cellulase productivity by insoluble solids recycling. Biotechnol Biofuels 2013;6:1-14; PMID:23298573; http://dx.doi.org/10.1186/1754-6834-6-5

[9] Weisenberg D, Kyriakides I, Agathos SN. White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol Adv 2003;22:161-87; PMID:14623049; http://dx.doi.org/10.1016/j.biotechnol.2003.08.011

[10] Eisenhauer N, Migunova VD, Ackermann M, Ruesch L, Scheu S. Changes in plant species richness induce functional shifts in soil nematode communities in experimental grassland. Plos One 2011;6:e24087; PMID:21909412; http://dx.doi.org/10.1371/journal.pone.0024087

[11] Mao X, Huixin L, Long M, Feng HU. Effects of bacteria-fecting nematode at its different density on bacterial number, bacterial activity and soil nitrogen mineralization. J Appl Ecol 2005;16:1112-6.

[12] Roos R, Mccag AE, Griffith BS, Prosser JJ. Impact of protozoan grazing on bacterial community structure in soil microcosms. Appl Environ Microbiol 2002;68:6094-105; PMID:12450833; http://dx.doi.org/10.1128/AEM.68.12.6094-6105.2002

[13] Lednická D, Mergaert J, Cnockaert MC, Swings J. Isolation and identification of cellulolytic bacteria involved in the degradatio of natural cellulolic fibres. Syst Appl Microbiol 2000;23:292-9; PMID:10930083; http://dx.doi.org/10.1016/S0702-23-200(0)(0)80017-X

[14] Poll J, Marhan S, Haase S, Hallmann J, Kandelé E, Ruess L. Low amounts of herbivory by root-knot nematodes affect microbial community dynamics and carbon allocation in the rhizosphere. Fems Microbiol Ecol 2007;62:268-79; PMID:17916076; http://dx.doi.org/10.1111/j.1576-6941.2007.00383.x

[15] Florencio C, Couri S, Farinas CS. Correlation between agar plate screening and solid-state fermentation for the prediction of cellulase production by Trichoderma strains. Petroleum Drilling Techniques 2011;41:100-5.

[16] Liu JM, Xin XJ, Li CX, Xu JH, Bao J. Cloning of thermostable cellulase genes of clostridium thermocellum and their secretive expression in bacillus subtilis. Appl Biochem Biotechnol 2012;166:525-62; PMID:22101447; http://dx.doi.org/10.1007/s12100-011-9456-z

[17] Tanskul S, Amornthatree K, Jaturonlak N. A new cellulose-producing bacterium, Rhodococcus sp MI 2: Screening and optimization of culture conditions. Carbohydr Polym 2013;92:421-8; PMID:23218315; http://dx.doi.org/10.1016/j.carbpol.2012.09.017

[18] Maurya DP, Singh D, Prapat D, Maurya JP. Optimization of solid state fermentation conditions for the production of cellulase by Trichoderma reesei. J Environ Biol 2012;33:5-8; PMID:23033636

[19] Sivakumar S, Nityanandi D, Barathi S, Prabha D, Rajeshwari S, Son HK, Subhuraam CV. Selected enzyme activities of urban heavy metal-polluted soils in the presence and absence of an oligochaete, Lampito mauritii (Kimberg). J Hazard Mater 2012;227-228:179-84; PMID:22658212; http://dx.doi.org/10.1016/j.jhazmat.2012.05.030

[20] Schagger H, Jagow GV. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 1987;166:368-79; PMID:2449095; http://dx.doi.org/10.1016/0003-2697(87)90587-2

[21] Wang X, Peng Z, Sun X, Liu D, Chen S, Li F, Xia H, Lu T. The FPase properties and morphology changes of a cellulolytic bacterium, Sporocytophaga sp. JL-01, on decomposing filter paper cellulose. J Gen Appl Microbiol 2012;58:429-36; PMID:23337578; http://dx.doi.org/10.1323/jgam.58.429

[22] Gusakov AV, Kondratyeva EG, Sinitsyn AP. Comparison of two methods for assaying reducing sugars in the determination of carbohydrate activities. Int J Anal Chem 2011;26:283658-8; PMID:21647284

[23] Borgna JL. Requirements for reliable determination of binding affinity constants by saturation analysis approach. J Steroid Biochem Mol Biol 2004;92:419-33; PMID:15698547; http://dx.doi.org/10.1016/j.jsbmb.2004.08.004

[24] Reiken SR, Knob RJ, Briedis DM. Evaluation of intrinsic immobilized kinetics in hollow fiber reactor systems. Enzyme & Microb Technol 1990;12:736-42; PMID:1367479; http://dx.doi.org/10.1016/0141-0229(90)90144-F

[25] Karpe F, Hamsten A. Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE. J Lipid Res 1994;35:1311-7; PMID:7964192

[26] Cheng F, Li S, Li C, Shuang M, Zou L. Isolation, identification and cellulase production of a cellulolytic...
bacterium from intestines of giant panda. Acta Microbiologica Sinica 2012;52:1113-21; PMID:23236845

[27] Béguin P. Molecular biology of cellulose degradation. Ann Rev Microbiol 1990;44:219-48; http://dx.doi.org/10.1146/annurev.mi.44.100190.001251

[28] Chen YL, Xu CL, Xu XN, Xie H, Zhang BX, Qin HG, Zhou WQ, Li DS. Evaluation of predation abilities of Blattisocius dolichus (Acari: Blattisocidae) on a plant-parasitic nematode, Radopholus similis (Tylenchida: Pratylenchidae). Exp Appl Acarol 2013;60:289-98; PMID:23269565; http://dx.doi.org/10.1007/s10493-012-9650-x

[29] Duboisset J, Ferrand P, He W, Wang X, Rigneault H, Brasselet S. Thioflavine-T and Congo Red reveal the polymorphism of insulin amyloid fibrils when probed by polarization-resolved fluorescence microscopy. J Phys Chem B 2013;117:784-8; PMID:23289901; http://dx.doi.org/10.1021/jp309528f

[30] Farnet AM, Qasemian L, Peter-Valence F, Ruaudel F, Savoie JM, Ferré E. Capacity for colonization and degradation of horse manure and wheat-straw-based compost by different strains of Agaricus subrufescens during the first two weeks of cultivation. Bioreourc Technol 2012;131C:266-73; PMID:23357087

[31] Vaughan NE, Lenton TM. Interactions between reducing CO2 emissions, CO2 removal and solar radiation management. Philos Trans Royal Soc A Math Phys Eng Sci 2012;370:4343-64; PMID:22869802; http://dx.doi.org/10.1098/rsta.2012.0188

[32] Mandels M, Hontz L, Nystrom J, Lb LRL. Enzymatic hydrolysis of waste cellulose. Biotechnol Bioeng 2010;105:1-2; PMID:19937801; http://dx.doi.org/10.1002/bit.22603

[33] Gu W, Zhang F, Xu P, Xie K. Screening of two straw-cellulose degrading actinomycetes. Acta Microbiologica Sinica 2012;52:1085-93; PMID:23236842

[34] Aragone MR, Maurizi DM, Clara LO, Navarro Estrada JL, Asionce A. Pseudomonas mendocina, an environmental bacterium isolated from a patient with human infective endocarditis. J Clin Microbiol 1992;30:1583-4; PMID:1624580

[35] Reyes MP, Palutke WA, Wylin RF. Pseudomonas endocarditis in the Detroit Medical Center. 1969-1972. Medicine 1973;52:173-94; PMID:20407410; http://dx.doi.org/10.1097/00005792-197305000-00001

[36] Yen KM, Karl MR, Blatt LM, Simon MJ, Winter RB, Fausset PR, Lu HS, Harcourt AA, Chen KK. Cloning and characterization of a Pseudomonas mendocina KRI gene cluster encoding toluene-4-monooxygenase. J Bacteriol 1991;173:5315-27; PMID:1885512

[37] Guo W, Song C, Kong M, Geng W, Wang Y, Wang S. Simultaneous production and characterization of medium-chain-length polyhydroxyalkanoates and alginate oligosaccharides by Pseudomonas mendocina NK-01. Appl Microbiol Biotechnol 2011;92:791-801; PMID:21617928; http://dx.doi.org/10.1007/s00253-011-3333-0

[38] Anzai Y, Kim H, Park JY, Wakabayashi H, Oyaizu H. Phylogenetic affiliation of the pseudomonads based on 16 S rRNA sequence. Int J Syst Evol Microbiol 2000;50:1563-89; PMID:10939664; http://dx.doi.org/10.1099/00207713-50-4-1563

[39] Rousk J, Baath E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N. Soil bacterial and fungal communities across a pH gradient in an arable soil. Isme J 2010;4:1340-51; PMID:20445636

[40] Kim S, Sun HH, Cho K, Lee I, Yoo G, Kang H. Effects of elevated CO2 and Pb on the microbial community in the rhizosphere of Pinus densiflora. J Microbiol 2012;50:895-901; PMID:23274974; http://dx.doi.org/10.1007/s12275-012-2207-1