

Newly Isolated Cellulose-Degrading Bacterium *Achromobacter xylosoxidans* L2 Has Deinking Potential

Yahua Li,^a Li Zhang,^a Hongquan Xian,^a and Xiaomei Zhang^{b,*}

Compared with conventional chemical deinking, enzymatic deinking is a more environmentally compatible alternative for processing recovered paper. In this study, a bacterial isolate identified as *Achromobacter xylosoxidans* (designated as L2) was found to be a promising candidate for a paper deinking process. It showed endoglucanase, β -glucosidase, endoxylanases, and filter paper degrading activities (FPase) with the maximal values of 2.60, 2.57, 3.08, and 24.58 U/mL, respectively. The optimal temperature and pH for FPase were determined to be 44 °C and pH 7.6. After two days of incubation with L2 at 130 rpm and 30 °C, the waste newspaper strips (0.5 cm × 5 cm) were disintegrated to debris with the average size of 3.5 mm (while that of the untreated without inoculation of L2 was 5.7 mm). The final brightness of the regenerated handsheets after incubation with L2 increased 3.7 units (31.4% ISO). The isolate and its complete lignocellulosic enzymes could be applied as deinking agents in the recycling of waste paper.

Keywords: *Achromobacter xylosoxidans*; Brightness; Deinking; Paper recycling

Contact information: a: Shandong Provincial Key Laboratory of Applied Mycology, College of Life Science, Qingdao Agricultural University, Qingdao, 266109, China; b: College of Resources and Environment, Qingdao Agricultural University, Qingdao, 266109, China; *Corresponding author: m0620@126.com

INTRODUCTION

Paper manufacturing is a continuously growing industry. At present, the raw materials needed for paper production comes from forests, agro residues, and waste paper. With forest resources decreasing and with increasing demands for agricultural residues for other applications, the recycling of used paper can represent a renewable fiber resource for paper manufacture both at home and abroad (Vyas and Lachke 2003).

As an important step in paper recycling, deinking involves the removal of the ink particles from the fiber surface. Chemical agents are applied in the conventional ink removal process, such as chlorine, sodium hydroxide, and hydrogen peroxide, *etc.*, which are costly; and they generate hazardous waste to the environment. Lignocellulosic enzymes such as cellulases and xylanases have been reported to be the most effective as both enzymes act upon the pulp fibers to facilitate the release of attached inks (Kirk and Jeffries 1996; Patrick 2004). Since it is environmentally friendly, enzymatic deinking has been reported as a potentially efficient solution for paper recycling (Prasad *et al.* 1992).

However, the substrate specificity of lignocellulosic enzymes is quite high. For example, endoglucanases (EC 3.2.1.4) degrade cellulose chains randomly, and endo- β -1,4-xylanases (EC 3.2.1.8) only act on the β -(1,4)-glycosyl bonds connecting D-xylose units in the xylans. The high specificity of a given lignocellulosic enzyme, in many cases, results in very low deinking efficiencies. Enzymatic deinking is more effective when combining two or more enzymes, as they often act synergistically.

Therefore, it is very important to find microorganisms that can secrete multiple enzymes. In this study, an isolate identified as *Achromobacter xylosoxidans* was found to exhibit great potency in the degrading of polysaccharides and in the deinking of waste paper. It shows great potential in paper manufacturing industry.

EXPERIMENTAL

Materials

Strain and chemicals

The bacterial strain used in this study was isolated as described later in the Methods section ("Screening and identification of the isolate"). Carboxymethylcellulose (CMC) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and xylan (beechwood) was obtained from Megazyme (Bray, Ireland). All other chemicals used in this study were of reagent grade and purchased from Sangon Biotech Co., Ltd. (Shanghai, China).

Culture medium

The medium used in the enrichment of the bacteria with cellulose-degrading and deinking capacities is described by Walker and Warren (1938) with the addition of 2.0% (w/v) filter paper (Whatman No. 1, 0.5 cm × 5 cm) as the sole carbon source (pH 7.0). The selective medium contained: 15.0 g/L CMC; 1.0 g/L yeast extract; 1.0 g/L NH₄NO₃; 0.5 g/L MgSO₄·7H₂O; 1.0 g/L KH₂PO₄; 1.0 g/L Congo red; and 20 g/L agar. For cellulase production, filter paper (2.0 g/L) was added in the Mendels salts as the fermentation medium, while for endoxylanase production, the filter paper was replaced by the same amount of xylan as the sole carbon source (Zhou *et al.* 2008).

Methods

Screening and identification of the isolate

The soil sample was collected from the forest in Qingdao Mount Laoshan in Shandong Province, China (120.5 E, 36.1 N). It was first incubated in the enrichment medium for 72 h at 160 rpm and 30 °C; after that, three times of subculturing of microbes was conducted, and 0.1 mL of the culture was then spread on the CMC-Congo-red agar plates. Colony's hydrolysis ability was preliminarily assessed by the diameter ratio of the hydrolytic circle to the colony (H/C). The colony with the largest ratio was chosen for further analysis. Routine identification of the isolate was performed in accordance to Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbons 1974).

Genomic DNA of the bacterial strain was extracted using a Bacterial DNA Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) for molecular identification. Primer sets for the 16S rRNA gene fragment were 27 F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492 R (5'-TACGGTTACCTTGTTACGACTT-3') (Lane 1991). The amplification program used was: 94 °C for 5 min; 34 cycles of 94 °C and 0.5 min; 53 °C and 0.5 min; 72 °C and 1.5 min; and extension at 72 °C for 10 min. The PCR product was out-sourced for sequencing at Sangon after it has been purified and quality checked. Resulting sequences were analyzed using the BLAST program in NCBI (<http://www.ncbi.nlm.nih.gov/>) (Xian 2017). The multiple sequence alignment was carried out using CLUSTAL W (<http://www.clustal.org/clustal2/>) (Zhang 2017), and the neighbor-joining phylogenetic tree was performed using MEGA 4.0 program as described by Tamura *et al.* (2007).

To further delineate the strain from closely related species, the housekeeping genes of *gyrB*, which encodes the DNA gyrase B subunit, were amplified and sequenced. The primers of *gyrB*1F (5'-CAYGCNGGNGGNAARTTYGA-3') and *gyrB*2R (5'-CCRTCACRTCNGCRTCNGTCAT-3') were chosen; the PCR conditions were: 94 °C, and 4 min; 35 cycles of 94 °C for 0.5 min; 52 °C and 0.5 min; and 72 °C and 1.5 min; and 1 cycle of 72 °C for 10 min (Martineau *et al.* 2001). Sequence blasting and phylogeny analysis were conducted using the same method as described earlier.

Factors influencing A. xylooxidans L2 growth and enzyme production

Culture flasks containing 100 mL of the fermentation medium inoculated with *A. xylooxidans* L2 were incubated at 30 °C with a shaking speed of 130 rpm. Cell growth was monitored for 12, 24, 36, 48, 60, 72 and 84 h. The supernatant of the zymotic liquid after centrifugation was used in the determination of the enzymatic activities on filter paper, CMC, salicin, and xylan as described by Chatterjee and Vining (1982) and by Wang *et al.* (1997). Enzymatic activities were defined as micromoles of reducing sugars formed per minute per milliliter of enzyme solution (U/mL); the relative activity was calculated by dividing each activity value with the maximum at the same environmental conditions.

To evaluate the effect of temperature on bacterial growth and enzyme production, culture flasks were incubated at 20, 25, 30, 35, and 40 °C for 48 h with a shaking speed of 130 rpm. The effect of pH was evaluated by cultivating strains at 30 °C for 48 h in the fermentation media that had been adjusted to the pH values of 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 beforehand with NaOH or HCl at room temperature. To detect the substrate profile, CMC was equivalently substituted by microcrystalline cellulose (Avicel), cotton and filter paper. The appropriate inoculum concentration was determined at 2, 4, 6, 8, 10, 12 and 14% of the total volume of the broth. The culture was incubated at 30 °C and 130 rpm for 48 h. Activities of FPase were determined, and cell growth was quantified by measuring the culture's optical density at 600 nm. All the growth experiments were conducted in triplicate.

Effects of temperature and pH on FPase

Impact of temperature on FPase activities was carried out at the optimum pH of 8.0 in the temperature range of 30 to 70 °C at 10 °C increments. The effect of pH on enzymatic activities was determined at the optimal temperature of 40 °C using three buffers (sodium citrate for pH 4.0 to 6.0, phosphate sodium for pH 6.0 to 8.0, and Tris-HCl for pH 8.0 to 9.0). All experiments were performed in triplicate, and the mean values were fitted through the Gaussian fit function in Origin Pro 8.5 software.

Deinking trial

The school newspaper of Qingdao agricultural university was applied in the deinking trial. The newspaper was printed using a flexographic method with flexographic ink, and the average weight per area was 50 g/m². The paper was first shredded into 0.5 cm × 5 cm strips, and then dipped into the Mendels salts with the amount of 2% (w/v). After sterilization, *A. xylooxidans* L2 was inoculated (4%, v/v) and cultivated in the medium-containing flasks at 130 rpm and 30 °C, and the untreated without inoculation of L2 run under identical conditions (130 rpm, 30 °C). After 2 days of fermentation, the morphology of the short fibers was observed, the sizes of the debris and the brightness of the pulp were measured.

Fiber morphology was observed by using a scanning electron microscope (JEOL 7500F, Oxford, Tokyo, Japan). The SEM voltage and distance was 15 kV and 10 mm, respectively. Paper samples were first washed three times with deionized water, air-dried, placed onto a bar, mounted with silver ribbon, and sprayed with gold (Hitachi S-4800, Hitachi, Tokyo, Japan). Fiber morphology was then observed at given magnification values.

For the debris size distribution analysis, a 4 mL sample of the zymotic liquid with debris was taken and transferred to petri dishes. The dishes with evenly spread liquid were placed in an oven at 50 °C overnight. The sizes of each piece were measured after it was dry, and the size distribution was evaluated by Gaussian nonlinear curve fitting.

To evaluate the deinking efficiency, a volume of 5 mL zymotic liquid was first centrifuged at 3000 rpm for 5 min. The supernatant was observed by an inverted microscope (CKX41, Olympus, Tokyo, Japan), and the rest was filtered using a screen (0.1 mm openings). The pulp retained on the screen was dried at 60 °C overnight. The brightness (% ISO) was measured at different places on the handsheets using a brightness and opacity tester (YQ-Z-48A, Qingtong, Hangzhou, China) with the directional reflectance at 457 nm, and values were expressed as the average percent value.

RESULTS AND DISCUSSION

Identification of the Isolate

A strain designated as L2 that has the largest H/C value of 11.2 was isolated from the CMC-Congo-red agar plate. The isolate was baculiform with the dimensions of 1.0 to 1.5 μm \times 0.5 to 0.7 μm . It was aerobic and Gram-negative with no endospores. Positive activities of catalase, oxidase, esterase, and urease were detected, and it also showed activities on nitrate, xylose and ribose. The taxonomic properties of isolate L2 (listed in Table S1) indicated that it should be phenotypically classified in the genus of *Achromobacter*.

The whole lengths of the 16S rRNA (accession no. KY486785) and the *gyrB* (accession no. KY486780) genes of the isolate were 1425 bp and 1143 bp, respectively. The phylogenetic trees that were constructed (Fig. 1) implied that the isolate L2 was clearly clustered into the clade of the genus *Achromobacter*, and was most closely related to the species of *xylosoxidans* with similarity values of 99.5 and 99.3%, respectively. Combination of the above morphological and phylogenetic analyses revealed that the isolate had the highest sequence similarities to that of *Achromobacter xylosoxidans*, and it was named as *Achromobacter xylosoxidans* L2.

Enzyme Production and Catalytic Properties

A. xylosoxidans L2 showed the highest growth rate at 30 °C and pH 7.0 with the optimal inoculum concentration of 12% (Fig. S1 a-c, see in Appendix). CMC was a suitable substrate for harvesting the biomass, but when it concerned to higher enzymatic activities, filter paper was found to be more suitable (Fig. S1d). The cellulose-degrading enzyme system of strain L2 was comprehensive, including FPase, endoglucanase, β -glucosidase and endoxylanase (Fig. 2a), and the highest activities were 24.58, 2.60, 2.57 and 3.08 U/mL, respectively.

Since it was first isolated in 1971, *A. xylosoxidans* has been widely studied (Yabuuchi 1973; Fabbri *et al.* 1987; De *et al.* 2007; Almuzara *et al.* 2010). Some

Achromobacter species were identified in the cellulose-degrading bacterial communities through the 16S rRNA pyrosequencing method (Dumova and Kruglov 2009). It was reported that an isolate of *Achromobacter* spp. acted synergistically with cellulolytic microbes of *Cytophagales* by producing β -glucosidase; however, the isolate itself showed no cellulolytic activities (Chen *et al.* 2015). As far as we know, the current investigation is the first report on the cellulolytic activities of *Achromobacter*, which is noteworthy in understanding the ecological roles of the genus (Dumova and Kruglov 2009; Yang *et al.* 2011; Talia *et al.* 2012). The optimal temperature and pH for FPase were determined to be 44 °C and pH 7.6 (Fig. 2b and 2c), which indicated that it was mesophilic and near-neutral.

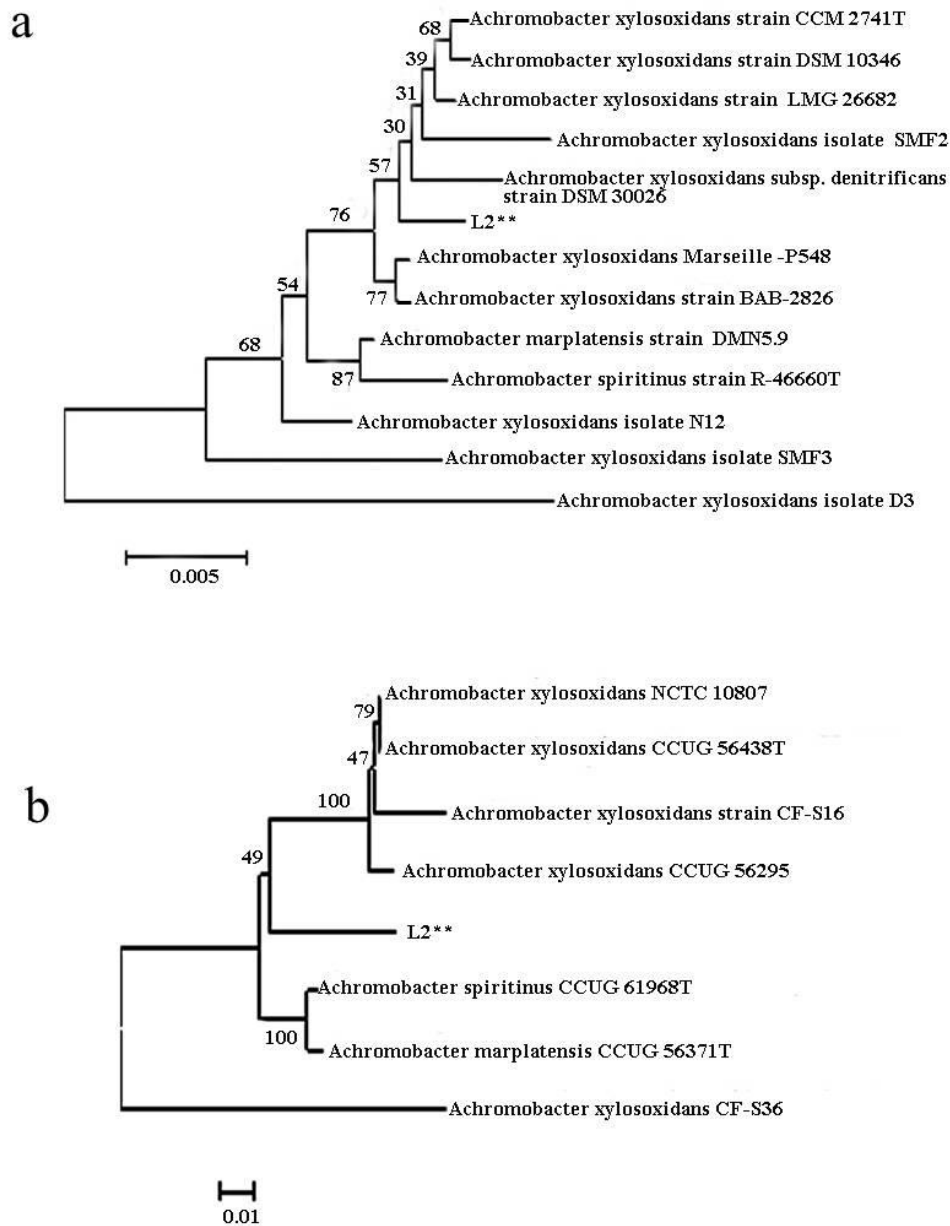


Fig. 1. Phylogenetic trees based on the 16S rRNA (a) and *gyrB* (b) gene sequence alignments. Bootstrap values expressed as a percentage of 100 replications were given at the branching points.

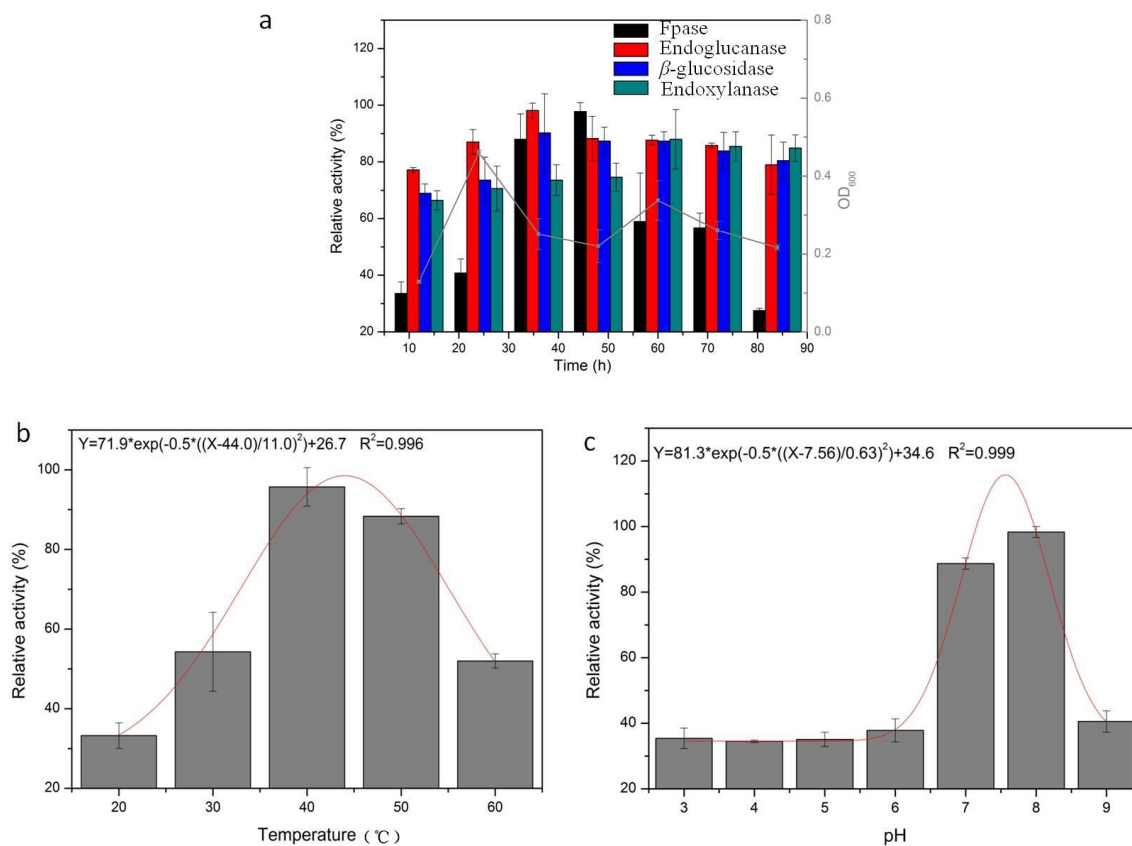


Fig. 2. Enzyme production and catalytic properties of *A. xylosoxidans* L2. (a) Changes of the enzyme activities and the growth of *A. xylosoxidans* L2 over time. Effects of temperature (b) and pH (c) on FPase. Relative activities were calculated by dividing each activity value with the maximum at the same condition, and results were expressed as the mean value \pm standard deviation based on three replicates ($n = 3$). Biomass changes in (a) were represented in gray polylines, and the red lines in (b) and (c) were the nonlinear curve fitting of the mean values through the Gaussian fit function in Origin Pro 8.5 software.

Deinking Capacity

After incubation with *A. xylosoxidans* L2 for 2 days, the waste newspaper was disintegrated into pulp, whereas the control was unchanged (Fig. S2). The morphology of the fibers as imaged by SEM confirmed that the fiber surface of the control was comparatively smooth with no sign of fibrillation, while that of the treated was rough with grooves, ridges and cracks (Fig. 3). The photographs (Fig. 3b, 3d) demonstrated that the treated modified the fiber surface by swelling and peeling, making the fiber surface more heterogeneous with small microfibrils. The difference about the particle size of the debris was obvious (Fig. 4a and 4c, respectively). The size of the debris after treatment ranged from 1.0 to 7.0 mm with the majority being about 3.5 mm, whereas the untreated particle size ranged from 4.0 to 10.0 mm with the majority being about 5.7 mm (Fig. 4d and 4b, respectively).

After centrifugation, the supernatant of the zymotic liquid of the waste newspaper showed more fibers but fewer ink specks than that of the untreated (Fig. 5a and 5d) when observed using an optical microscope.

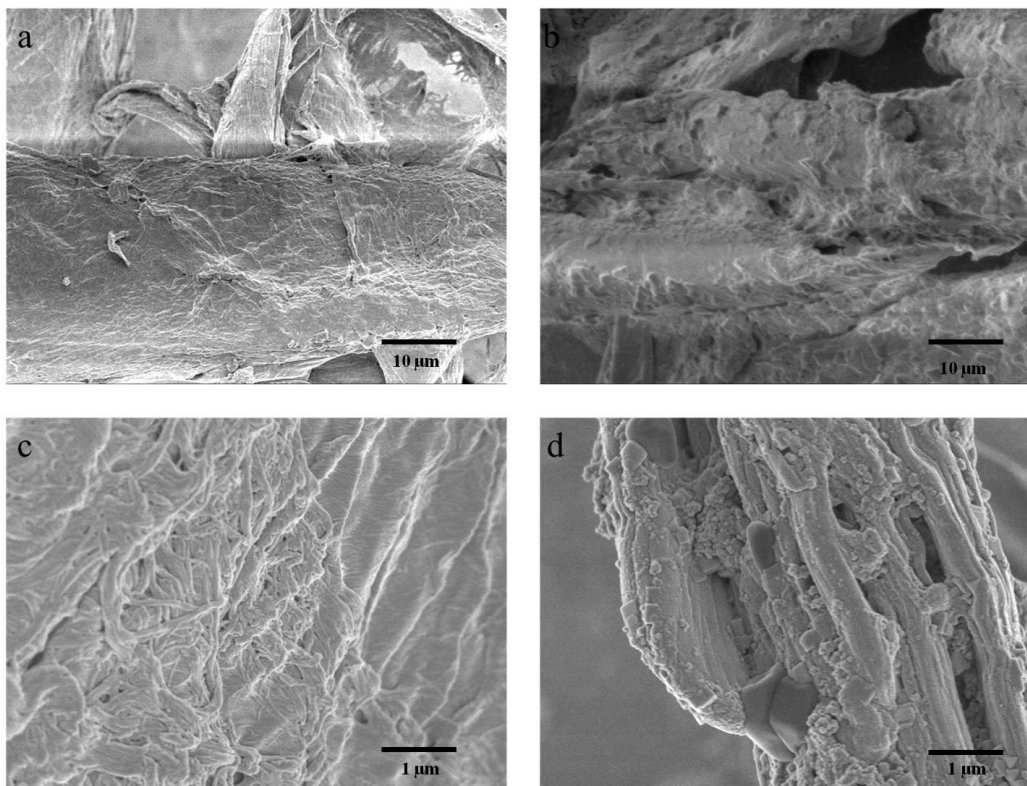


Fig. 3. SEM images to characterize fiber morphology. The left two (a and c) were on waste papers without treatment, and the right (b and d) were on waste papers incubated with *A. xylooxidans* L2.

Compared with the regenerated handsheets without inoculation of L2 (Fig. 5b), there were less debris visible on the paper surface of the treated sample (Fig. 5e), and the final brightness increased 3.7 units, reaching 31.4% ISO brightness. This result was consistent with previous reports that there were averagely 3 to 4 units of increase in newspaper brightness after enzymatic deinking (Morkbak and Zimmermann 1998; Lee *et al.* 2011; Ibarra *et al.* 2012). Meanwhile, the filtrate of the zymotic liquid inoculated with *A. xylooxidans* L2 was noticeably blacker and thicker after filtering (Fig. 5c and 5f). The dry mass of the filtrate after centrifugation was 0.2667 g/100 mL for the treatment, while that of the untreated was only 0.0467 g/100mL. The results above indicated that, L2 had great potency in peeling ink dots off.

As the carbohydrate hydrolases involved in paper deinking, endoglucanase and/or endoxyylanase seem to play important roles. Endoglucanases were considered to attack the less-ordered cellulose randomly, leading to fiber wall swelling and loosening of short fibers, and consequently facilitating the ink detachment (Gübitz *et al.* 1998; Vyas and Lachke 2003). Endoxylanases dislodge inks through hemicellulose hydrolysis on the fiber surface. Anyway, combination of the two activities promoted deinking efficiencies (Gübitz *et al.* 1998; Lee *et al.* 2007). In this sense, the comprehensive enzyme system of carbohydrate hydrolase possessed by L2 explained its capacity in paper deinking. Besides, neutral to basic enzymes that could better improve the brightness and cleanliness of the recycled fibers (Bhat 2000) further ensured *A. xylooxidans* L2 a promising candidate in paper recycling industrial. Future reports of the application of this bacterium and the enzymes that it produces are under investigation. The results from these studies will be communicated in future reports.

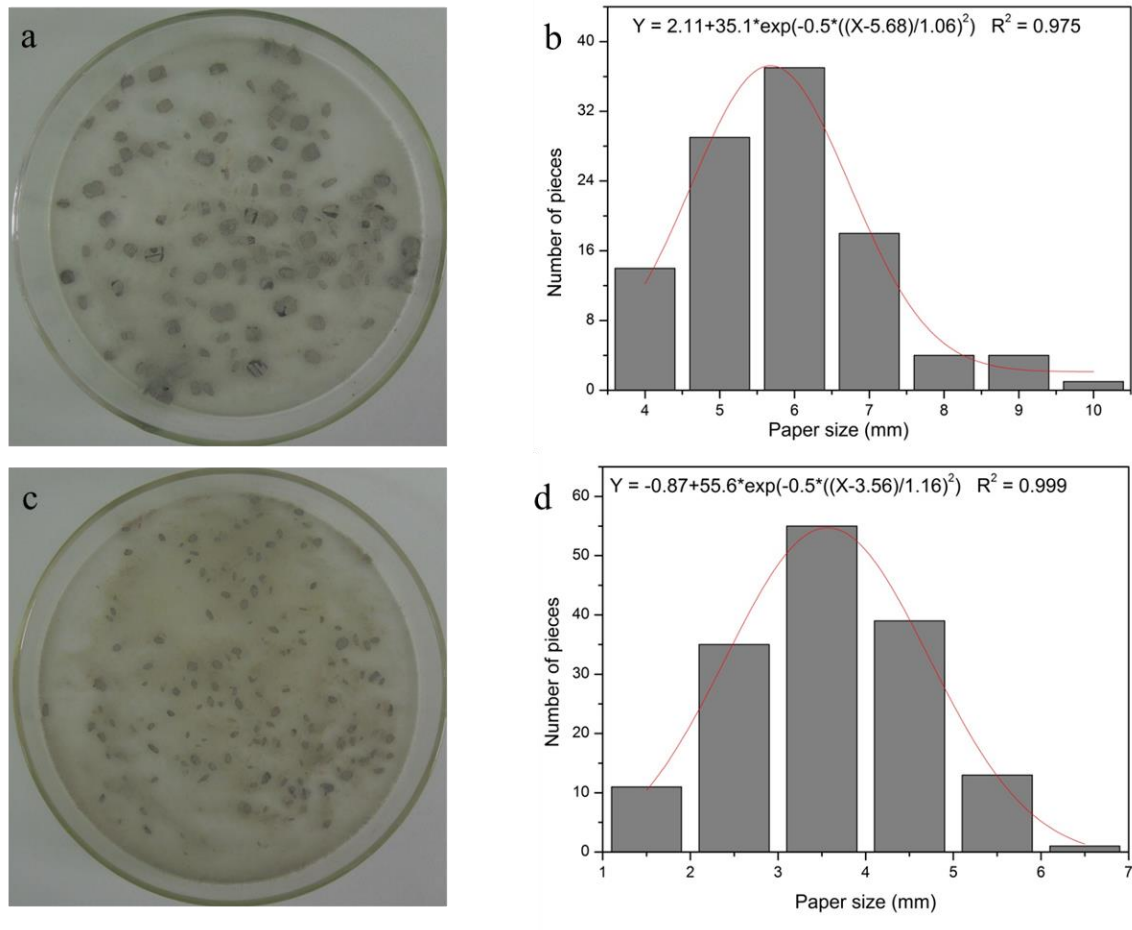


Fig. 4. Degradation capacity of L2 with waste newspapers. Dishes containing waste paper without (a) and with inoculation of L2 (c), and the corresponding debris size distribution (b and d). Debris was obtained from a volume of 4 mL of the zymotic liquid without or with incubation of L2 at 130 rpm and 30 °C for 2 days. Origin 8.5 software was used to analyze particle size distribution using a Gaussian distribution fit.

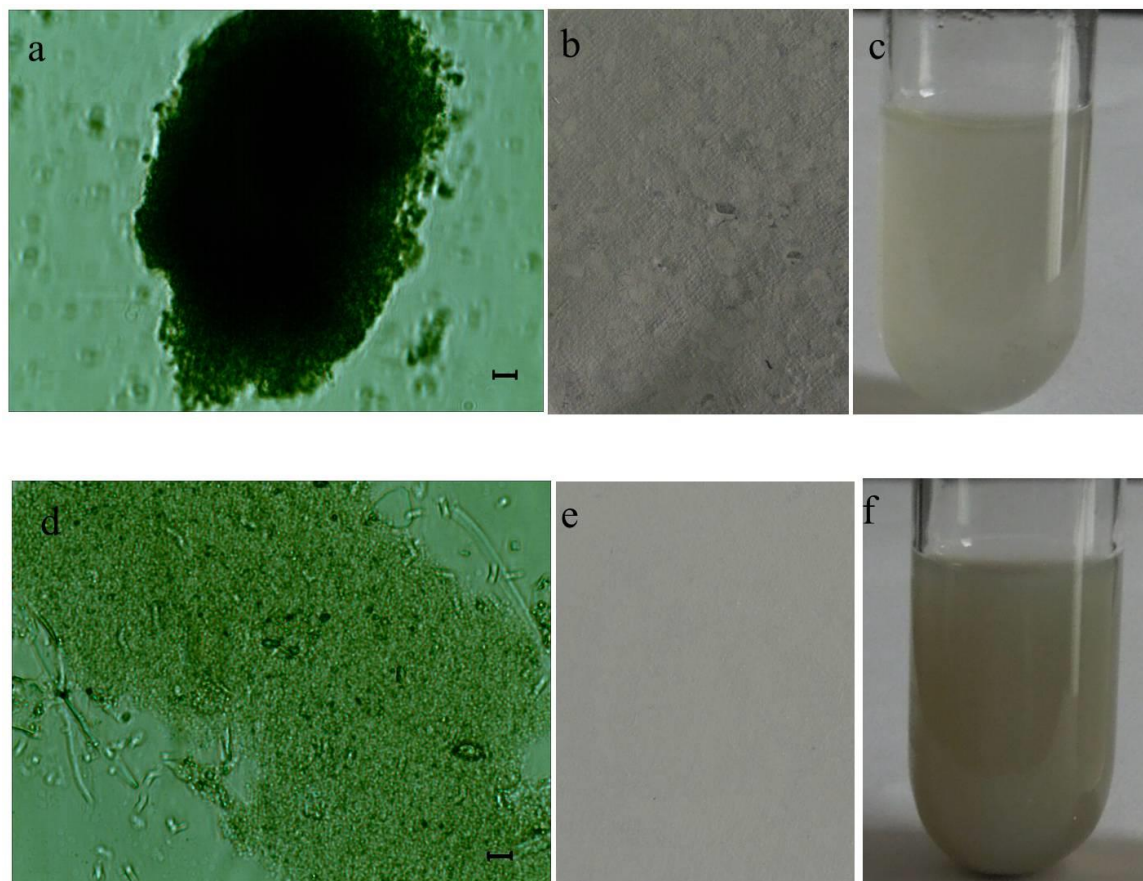


Fig. 5. Deinking treatment with *A. xylosoxidans* L2. Microscopic images (magnification 400 fold) of the fibers and ink particles left in the supernatant of the fermentation broth (a and d); (b) and (e) were the corresponding handsheets made from the pulp after filtering through a screen (0.1 mm openings); (c) and (f) were the filtrates collected. The upper (a, b and c) was the untreated, and the lower (d, e and f) was the results after incubation with L2 for 2 days at 130 rpm and 30 °C. The bars marked in (a) and (d) were 10 μ m.

CONCLUSIONS

1. A species of *Achromobacter*, which was identified as *Achromobacter xylosoxidans* (designated as L2), was isolated.
2. The strain was cellulolytic, and this is the first report regarding its ability in the degradation of polysaccharides.
3. *A. xylosoxidans* has potential use in deinking waste newsprint for paper manufacturing industrial.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (No. 31600084), the Key Research and Development Program of Shandong Province (2018GNC110024), and the High Level Talent Fund Project of Qingdao Agricultural

University (1117008 and 1113342). The support from all of these groups is gratefully appreciated.

REFERENCES CITED

- Almuzara, M., Limansky, A., Ballerini, V., Galanternik, L., Famiglietti, A., and Vay, C. (2010). "In vitro susceptibility of *Achromobacter* spp. isolates: Comparison of disk diffusion, Etest and agar dilution methods," *Int. J. Antimicrob. Agents* 35, 68-71. DOI: 10.1016/j.ijantimicag.2009.08.015
- Bhat, M. K. (2000). "Cellulases and related enzymes in biotechnology," *Biotechnol. Adv.* 18, 355-383. DOI: 10.1016/S0734-9750(00)00041-0
- Buchanan, R. E., and Gibbons, N. E. (1974). *Bergey's Manual of Systematic Bacteriology*, The Williams & Wilkins Company, Baltimore, MD, USA.
- Chatterjee, S., and Vining, L. C. (1982). "Glucose suppression of β -glucosidase activity in a chloramphenicol-pro," *Can. J. Microbiol.* 28, 593-599.
- Chen, X., Wang, Y., Yang, F., Qu, Y., and Li, X. (2015). "Isolation and characterization of *Achromobacter* sp. CX2 from symbiotic *Cytophagales*, a non-cellulolytic bacterium showing synergism with cellulolytic microbes by producing β -glucosidase," *Annu. Microbiol.* 65, 1699-1707. DOI: 10.1007/s13213-014-1009-6
- De, B. F., Schelstraete, P., Van, D. S., Haerynck, F., and Vaneechoutte, M. (2007). "*Achromobacter xylosoxidans* in cystic fibrosis: Prevalence and clinical relevance," *J. Cyst. Fibros.* 6, 75-78. DOI: 10.1016/j.jcf.2006.05.011
- Dumova, V. A., and Kruglov, Y. V. (2009). "A cellulose-decomposing bacterial association," *Microbiol.* 78, 234-239. DOI: 10.1134/S0026261709020155
- Fabbri, A., Tacchella, A., Manno, G., Viscoli, C., Palmero, C., and Gargani, G. F. (1987). "Emerging microorganisms in cystic fibrosis," *Chemioterapia* 6, 32-37.
- Gübitz, G. M., Mansfield, S. D., Böhm, D., and Saddler, J. N. (1998). "Effect of endoglucanases and hemicellulases in magnetic and flotation deinking of xerographic and laser-printed papers," *J. Biotechnol.* 65, 209-215. DOI: 10.1016/S0168-1656(98)00130-8
- Ibarra, D. M., Concepción, M., Blanco, A., Martínez, A. T., and Martínez, M. J. (2012). "Enzymatic deinking of secondary fibers: cellulases/hemicellulases versus laccase-mediator system," *J. Ind. Microbiol. Biotechnol.* 39, 1-9. DOI: 10.1007/s10295-011-0991-y
- Kirk, T. K., and Jeffries, T. W. (1996). "Roles for microbial enzymes in pulp and paper processing," in: *Enzymes for Pulp and Paper Processing*, ACS Symposium Series, Vol. 655, Jeffries, T. W. and Viikari, L. (eds.), American Chemical Society, Washington, DC, pp. 2-14. DOI: 10.1021/bk-1996-0655.ch001
- Lane, D. J. (1991). "16S/23S rRNA Sequencing," in: *Nucleic Acid Techniques in Bacterial Systematics*, Stackebrandt, E. and Goodfellow, M. (eds.), John Wiley and Sons, New York, NY, pp. 139-156.
- Lee, C. K., Darah, I., and Ibrahim, C. O. (2007). "Enzymatic deinking of laser printed office waste papers: some governing parameters on deinking efficiency," *Bioresour. Technol.* 98, 1684-1689. DOI: 10.1016/j.biortech.2006.05.052
- Lee, C. K., Ibrahim, C. O., and Wan, D. (2011). "Pilot scale enzymatic deinking of mixed office wastepaper and old newspaper," *Bioresources* 6, 380923.

- Martineau, F., Picard, F. J., Ke, D., Paradis, S., Roy, P. H., Ouellette, M., and Bergeron, M. G. (2001). "Development of a PCR assay for identification of *Staphylococci* at genus and species levels," *J. Clin. Microbiol.* 39, 2541-2547. DOI: 10.1128/JCM.39.7.2541-2547.2001
- Morkbak, A. L., and Zimmermann, W. (1998). "Deinking of mixed office paper, old newspaper and vegetable oil based ink printed-paper using cellulase xylanase and lipases," *Prog. Pap. Recycl.* 7, 14-27.
- Patrick, K. (2004). "Enzyme technology improves efficiency, cost, safety of stickies removal program," *Paper Age* 120, 22-25.
- Prasad, D. Y., Heitmann, J. A., and Joyce, T. W. (1992). "Enzyme deinking of black and white letterpress printed newsprint waste," *Prog. Pap. Recycl.* 1, 21-30.
- Talia, P., Sede, S. M., Campos, E., Rorig, M., Principi, D., Tosto, D., Hopp, H. E., Grasso, D., and Cataldi, A. (2012). "Biodiversity characterization of cellulolytic bacteria present on native Chaco soil by comparison of ribosomal RNA genes," *Res. Microbiol.* 163, 221-232. DOI: 10.1016/j.resmic.2011.12.001
- Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007). "MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0," *Mol. Biol. Evol.* 24, 1596-1599. DOI: 10.1093/molbev/msm092
- Vyas, S., and Lachke, A. (2003). "Biodeinking of mixed office waste paper by alkaline active cellulases from alkalotolerant *Fusarium* sp.," *Enzyme Microb. Technol.* 32, 236-245. DOI: 10.1111/j.1525-1438.2006.00720.x
- Walker, E., and Warren, F. L. (1938). "Decomposition of cellulose by *Cytophaga*. I," *Biochem. J.* 32, 31-43. DOI: 10.1042/bj0320031
- Wang, G., Michailides, T. J., and Bostock, R. M. (1997). "Improved detection of polygalacturonase activity due to *Mucor piriformis* with a modified dinitrosalicylic acid reagent," *Phytopathol.* 87, 161-163. DOI: 10.1094/PHYTO.1997.87.2.161
- Xian, H. (2017). "NCBI," (<https://www.ncbi.nlm.nih.gov/>), accessed 28 Jan 2017.
- Yabuuchi, E. (1973). "Identification of *Pseudomonas* and related organisms," *Rinsho. Byori.* 21, 102-108.
- Yang, H., Wu, H., Wang, X., Cui, Z., and Li, Y. (2011). "Selection and characteristics of a switchgrass-colonizing microbial community to produce extracellular cellulases and xylanases," *Bioresour. Technol.* 102, 3546. DOI: 10.1016/j.biortech.2010.09.009
- Zhang, X. M. (2017). "Multiple alignment of nucleic acid and protein sequences," (<http://www.clustal.org/clustal2/>), accessed 12 Feb 2017.
- Zhou, J., Wang, Y. H., Chu, J., Zhuang, Y. P., Zhang, S. L., Yin, P. (2008). "Identification and purification of the main components of cellulases from a mutant strain of *Trichoderma viride* T 100-14," *Bioresour. Technol.* 99, 6826-6833. DOI: 10.1016/j.biortech.2008.01.077

Article submitted: September 8, 2018; Peer review completed: December 15, 2018;
Revised version received and accepted: January 29, 2019; Published: February 1, 2019.
DOI: 10.15376/biores.14.1.2256-2268

APPENDIX

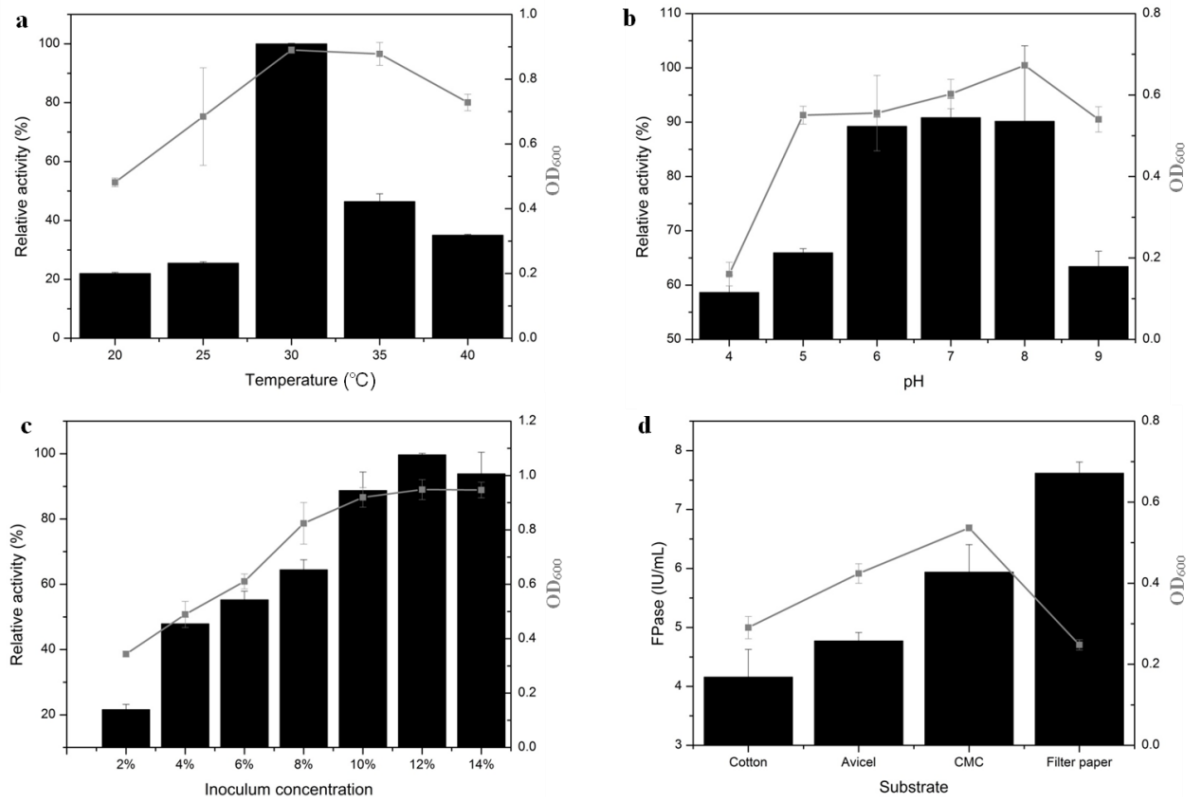


Fig. S1. Effects of temperature (a), pH (b), inoculum concentration (c) and substrates (d) on FPase and growth of *A. xylooxidans* L2. The gray polylines indicate changes of the biomass. Each data was the mean value of three replicates.

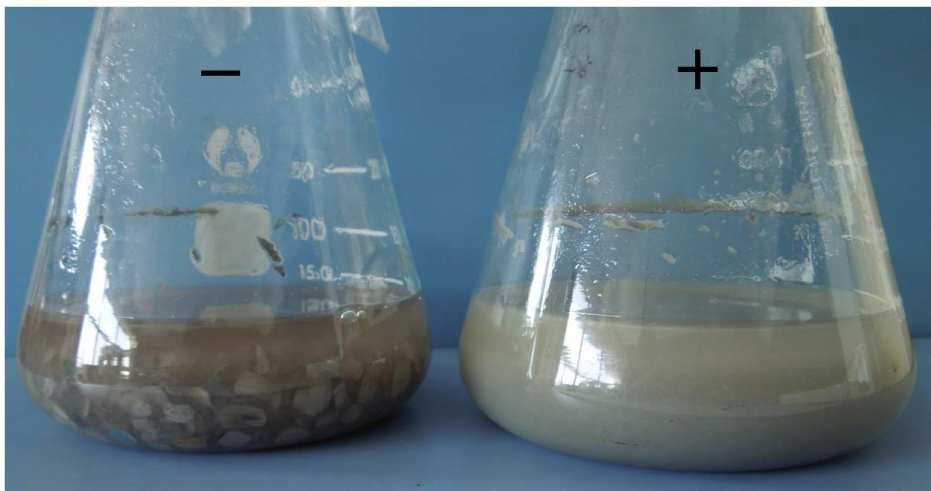


Fig. S2. Paper pulp without (-) or with (+) incubation of *A. xylooxidans* L2 at 130 rpm and 30 °C for 2 days. Waste newspaper was scissored into a size of approximately 0.5×5 cm² beforehand, and it was immersed in basic medium with the ratio of 2% (w/v).

Table S1. Detection of Phenotypic Characteristics of *A. xylooxidans* L2

Features	Results	Features	Results
Catalase	+	Xylose	+
Oxidase	+	Glucose	-
Esterase	+	Lactose	-
Urease	+	Mannose	-
H ₂ S production	-	Ribose	+
Nitrate	+	Maltose	-
Sorbol	-	Sucrose	-
Mannitol	-	Rhamnose	-
Fructose	-		