Supporting information

Experimental Section

Strains and Plasmids

E. coli BL21 (DE3) (Novagen, USA) was used as an expression host. *E. coli* DH5α (Novagen, USA) was used to amplify plasmids. The pET30a (Novagen, USA) was used as an expression vector. The genome of *Trichoderma reesei* QM9414 (ATCC26921) and *Cellulomonas fimi* (ATCC 484) were used as templates to amplify endoglucanase and β-glucosidase encoding sequences, respectively. The culture medium and conditions of all strains were described previously [1].

Construction of co-expression plasmid and mutant library

The bicistronic operon (PcenA-bgl) was constructed by introducing an internal ribosome binding site ahead of the second gene (bgl), as described previously [1]. A random library of cenA-bgl mutants was constructed by error-prone PCR. The plasmid of pcenA-bgl was used as a template. To increase the efficiency of positive colonies, the forward primer for T7 promoter (5'-TAATACGACTCACTATAGGG-3') and the reverse primer for T7 terminator (5'-TGCTAGTTATTGCTCAGCGG-3') were used to amplify gene cenA-bgl. The PCR system contained 10 ng template plasmid (PcenA-bgl), 2.5 mM Mg^{2+},1× GC buffer I, 0.4 mM dNTP, 0.2 μM of each primer, 0.05 mM Mn^{2+} and 2.5 U of Taq polymerase in a total volume of 50 μl. The PCR reaction was carried out with *LA* Taq DNA polymerase with GC buffer (TakaRa) under the following conditions: 94 °C denaturation for 1 min, 30 cycles of 94 °C denaturation for 30 sec, 54 °C annealing for 30 sec and 72 °C extension for 2.5 min. The amplified PCR products were then digested with NspV and EcoRI, and ligated into expression vector pET30a digested with the same enzymes. The recombinants were transformed into the strain *E. coli* BL21 (DE3) using a heat pulse at 42 °C and grown on LB medium plates (yeast extract 5 g/l; peptone 10 g/l; NaCl 10 g/l) containing kanamycin (50 μg/ml). Nearly 3,000 colonies were obtained in each round of screening.

High-throughput screening for mutants with improved hydrolytic activity

The colonies were selected from mutant library and transferred to 96-well microtiter plates by sterilized toothpicks. The cells were incubated in 96-well microtiter plates for 12-16 h at 37 °C. An equivalent culture from each well was added into the corresponding fresh wells on another 96-well microtiter plate. Cells were grown for 2-3 h at 37 °C to an optical density. Subsequently, isopropyl-beta-D-thiogalactopyranoside (IPTG) was added to the culture medium at a final concentration of 0.1 mM to induce protein expression, and growth was continued for another 4 h. The cells were collected by centrifugation at 4000 g for 10 min and then were frozen at -80 °C overnight. *E. coli* cells were lysed with lysis buffer (Tris-HCl, 50 mM; $MgCl_2$, 10 mM; lysozyme, 0.5 mg/ml; DNase I, 2000 U/l; pH 8.0) at 37 °C for 1 h. The crude enzyme activity was assayed with Whatman No. 1 filter paper, which was conducted as follows: 7 mm disk of Whatman No. 1 filter paper and appropriate crude enzyme from corresponding 96-well microtiter plates in a 60 μl format assay (50 mM NaAc buffer, pH 4.8), according to the method described by Xiao *et al* [2]. The reactions were carried out at 40 °C for 30 min. The product of glucose was measured with a coupled glucose oxidase and peroxidase assay kit (Rsbio, China).

Subcloning of CenA and BGL from positive mutants

The cenA genes from mutants 1-B7, 2-A4 and 3-A12 were digested with NspV and KpnI, ligated into the expression vector pET30a digested with the same enzymes, and transformed into *E. coli* BL21 (DE3), generating 1-B7-CenA, 2-A4-CenA and 3-A12-CenA, respectively. The bgl genes from mutants 2-F6, 3-A12 and 3-H1 were digested with KpnI and EcoRI, ligated into pET30a digested with the same enzymes, and transformed into *E. coli* BL21 (DE3), generating 2-F6-BGL, 3-A12-BGL and 3-H1-BGL, respectively. The cenA gene from 3-A12-CenA was digested with NspV and KpnI, ligated into 3-H1-BGL digested with the same enzymes, generating the new plasmind of A12-H1.

Enzyme purification and characterization

The wild type and six mutants were inoculated into 5 ml of LB broth (50 μg/ml kanamycin), and grown overnight. This was then diluted into 50 ml LB broth (50 μg/ml kanamycin) at 37 °C until the optical cell density at 600 nm (OD600) reached 0.6-0.8. The growth was continued at 16 °C overnight after 0.1 mM IPTG was added. After cultivation, cells were harvested by centrifugation at 4000 g for 10 min, washed with PBS buffer and resuspended in 50 mM Tris-HCl (pH 8.0). The cells were lysed by sonication. The cell debris and the insoluble fraction was removed by centrifugation at 10,000 g for 10 min at 4 °C. All purifications were performed using ÄKTA purifier FPLC purification system and facilitated with the His Trap™ HP column (GE Healthcare, Sweden). Protein concentration was determined by the method of Bradford. Protein expression in all constructs was analyzed by 12 % SDS-PAGE. Filter paper activity was measured with purified enzyme, as described previously[1]. β-glucosidase activity toward D-cellobiose (Sigma) was determined by incubating appropriate enzyme in 0.6 ml 50 mM sodium phosphate buffer (pH 6.0) with 0.4 ml 15 mM D-cellobiose at 40 °C for 10 min. One unit of β-glucosidase activity was defined as the amount of enzyme required to produce 1 μmol glucose per min under the assay condition.

1. M. Liu and H. Yu, *Biochem. Eng. J.*, 2012, **69**, 204-210.
2. Z. Xiao, R. Storms and A. Tsang, *Biotechnol. Bioeng.*, 2004, **88**, 832-837.