Enhancing cellulase and hemicellulase production by genetic modification of the carbon catabolite repressor gene, creA, in Acremonium cellulolyticus

Tatsuya Fujii*, Hiroyuki Inoue and Kazuhiko Ishikawa

Abstract

Acremonium cellulolyticus is one of several fungi that offer promise as an alternative to Trichoderma reesei for use in industrial cellulase production. However, the mechanism of cellulase production has not been studied at the molecular level because adequate genetic engineering tools for use in A. cellulolyticus are lacking. In the present study, we developed a gene disruption method for A. cellulolyticus, which needs a longer homologous region length. We cloned a putative A. cellulolyticus creA gene that is highly similar to the creA genes derived from other filamentous fungi, and isolated a creA disruptant strain by using the disruption method. Growth of the creA disruptant on agar plates was slower than that of the control strain. In the wild-type strain, the CreA protein was localized in the nucleus, suggesting that the cloned gene encodes the CreA transcription factor. Cellulase and xylanase production by the creA disruptant were higher than that of the control strain at the enzyme and transcription levels. Furthermore, the creA disruptant produced cellulase and xylanase in the presence of glucose. These data suggest both that the CreA protein functions as a catabolite repressor protein, and that disruption of creA is effective for enhancing enzyme production by A. cellulolyticus.

Keywords: Cellulase; Hemicellulase; Catabolite repression; Acremonium cellulolyticus

Introduction

Lignocellulosic biomass is a promising material for use in biorefining because it contains a large amount of sugar in the form of cellulose and hemicellulose (Lynd 1996). Cellulase and hemicellulase are the two major families of enzymes that hydrolyze cellulose and hemicellulose (a lignocellulose) to monomeric sugars. Some filamentous fungi, such as Trichoderma reesei, secrete large amounts of cellulase and hemicellulase (Goyal et al. 1991; Krogh et al. 2004; Sehnem et al. 2006; Wen et al. 2005). The cellulases produced by fungi include three major groups of enzymes: endoglucanases, which randomly hydrolyze internal glycosidic linkages; cellobiohydrolases, which produce cellobiose from cellulose chain ends; and β-glucosidases, which convert cellobiose into glucose (Goyal et al. 1991).

The filamentous fungus, Acremonium cellulolyticus, which was isolated in 1982 from soil in Japan, is a cellulose-degrading organism (Yamanobe et al. 1987) and is one of several fungi that offer promise as an alternative to T. reesei for the industrial production of cellulase. A cellulase mixture produced by A. cellulolyticus is commercially sold as ‘Acremonium cellulase’ by Meiji Seika Pharma Co., Strains TN, C-1, and CF-2612, which are cellulase hyper-producing mutants, were isolated from the wild type strain Y-94 by random mutagenesis (Fang et al. 2009; Yamanobe et al. 2003). The enzymes from A. cellulolyticus reportedly produce glucose more rapidly from various lignocellulosic materials than the enzymes from T. reesei (Fujii et al. 2009). Over 40 reports or patents related to A. cellulolyticus have been published, making it one of the best characterized cellulase-producing organisms. Furthermore, a genomic database (unpublished data) and transformation system for A. cellulolyticus (Fujii et al. 2012) have been constructed by our group. We successfully overexpressed

* Correspondence: tatsuya.fujii@aist.go.jp
Biomass Refinery Research Center, National Institute of Advanced Industrial Science and Technology (AIST), 3-11-32 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-0046, Japan

© 2013 Fujii et al; licensee Springer. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
cellulase and hemicellulase genes in this organism and constructed a starch-inducible homologous expression system (Inoue et al. 2013; Kanna et al. 2011), thus making available genetic engineering tools suitable for *A. cellulolyticus*. However, the development of these tools is not sufficient because gene targeting, such as gene disruption by homologous recombination, is difficult in *A. cellulolyticus*. Several transcription factors have been reported as regulators of cellulase and hemicellulase gene expression in other filamentous fungi, e.g., XlnR/Xyr1 for genes encoding cellulase, hemicellulase, and accessory enzymes involved in xylan degradation in *Aspergillus niger* and *T. reesei* (Stricker et al. 2006; van Peij et al. 1998); Ace2 for cellulase genes in *T. reesei* (Aro et al. 2001); BglR for the β-glucosidase in *T. reesei* (Nitta et al. 2012); and AraR for the L-arabinose reductase gene in *A. niger* and *Aspergillus nidulans* (Battaglia et al. 2011). These transcription factors specifically regulate the expression of cellulase and hemicellulase genes. On the other hand, some transcription factors regulate a wide range of genes including cellulase and hemicellulase genes. These factors include CreA (Dowzer and Kelly 1989; Ilmen et al. 1996; Nakari-Setälä et al. 2009; Wang et al. 2013), which is involved in catabolite repression; AreA (Lockington et al. 2002), which is involved in nitrogen source assimilation; and the Hap complex (Tsukagoshi et al. 2001), which regulates various genes. Although a number of transcription factors involved in regulating cellulase and hemicellulase gene expression in other filamentous fungi have been analyzed, the regulation of these gene expressions in *A. cellulolyticus* has not been investigated. Because *A. cellulolyticus* is an industrially important fungal species, how cellulase and hemicellulase gene expression is regulated in this organism is crucial for the development of more efficient enzyme production methods.

In the present study, we cloned a putative creA gene from *A. cellulolyticus* that is highly similar to the creA genes of other filamentous fungi and then isolated a recombinant strain in which the creA gene was disrupted. The length of the homologous region was important for gene disruption. Growth of the creA disruptant on agar plates was slower than that of the control strain, and CreA protein was found to localize in the nucleus. The production of cellulase and xylanase by the creA disruptant was higher than that of the control strain at both the enzyme and transcription level. Furthermore, the creA disruptant produced cellulase and xylanase in the presence of glucose. These data suggest that the cloned putative creA gene encodes a catabolite repressor protein, and that disruption of creA leads to enhanced enzyme productivity.

**Materials and methods**

**Strains, cultures, and media**

The strains used in this study are listed in Table 1. *A. cellulolyticus* YP-4 (Inoue et al. 2013), which is a uracil auxotrophic strain derived from *A. cellulolyticus* Y-94 (Yamanobe et al. 1987) (FERM BP-5826), was maintained on potato dextrose agar (PDA) (Difco, Detroit, MI) plates containing 1 g/L of uracil and 1 g/L of uridine. The transformants were maintained on MM plates (1% glucose, 10 mM NH₄Cl, 10 mM potassium phosphate (pH 6.5), 7 mM KCl, 2 mM MgSO₄). For measurement of enzyme activity and gene expression, the strains were cultivated in 10 mL of basic medium (24 g/L of KH₂PO₄, 1 g/L of Tween 80, 5 g/L of (NH₄)₂SO₄, 1.2 g/L of MgSO₄·7H₂O, 0.01 g/L of ZnSO₄·7H₂O, 0.01 g/L of MnSO₄·6H₂O, 0.01 g/L of CuSO₄·7H₂O; pH 4.0) supplemented with 2 g/L of urea and 40 g/L of glycerol in 100-mL Erlenmeyer flasks at 30°C for 72 h on a rotary shaker at 230 rpm. The cells were then washed 3 times with saline, and aliquots of the washed cells were inoculated into 10 mL of basic medium supplemented with 4 g/L of urea and 10 to 50 g/L of carbon sources in 100-mL Erlenmeyer flasks which were then incubated at 30°C on a rotary shaker at 230 rpm. Cellulose (Solka Floc; Fiber Sales & Development, Urbana, OH), xylan (Birchwood xylan; SIGMA, St. Louis, MO), glucose or glycerol were used as the carbon source.

**Plasmid construction and fungal transformation**

The plasmids used in this study are listed in Table 1. Plasmids used for disrupting the creA gene were constructed by inserting DNA fragments carrying the 5’ and 3’ regions of creA into the upstream and downstream regions of the pyrF gene in pbs-pyrF (Fujii et al. 2012). The DNA fragments carrying the 5’ regions fused with appropriate restriction sites were amplified using the primers creA 1000u-f and creA 1000u-r (for pDCre1000) or creA 2500u-f and creA 2500u-r (for pDCre2500) (Table 2), digested with EcoRI and Sall, and ligated with pBS-pyrF which had already been spliced with the same restriction enzymes. The 3’ region of each gene was amplified using the primers creA 1000d-f and creA 1000d-r (for pDCre1000) or creA 2500d-f and creA 2500d-r (for pDCre2500) (Table 2), digested with XbaI and NotI, and inserted into the same restriction sites of the resulting plasmids to generate pDCre1000 and pDCre2500. These plasmids were digested with NotI before using fungal transformation. The pCreGFP plasmid used for production of the CreA-green fluorescent protein (GFP) fusion was constructed as follows. The DNA fragment encoding GFP was amplified from pGFPuv (Takara bio, Otsu, Japan) using the primers gfp-f and gfp-r (Table 2), and then digested with KpnI and EcoRI. The creA DNA fragment including the promoter region
ments were inserted into the Eco
plasmids used in this study
pCreGFP Amp r PyrFr; pbs-pyrF derivative
pDCre1000 Amp r PyrFr; pbs-pyrF derivative
pbs-pyrF Amp r PyrFr; pBluescript KS(+) Plasmid
YCreGFP YP-4 prototrophic transformant
YPyrF YP-4 prototrophic transformant

Single-stranded cDNA was synthesized and then real-time quantitative RT-PCR was amplified using primers creAN-f and creAN-r, and then digested with Apal and KpnI. The resulting fragments were inserted into the EcoRI and Apal sites of pbs-pyrF to generate pCreGFP. Fungal transformation was carried out as described previously (Fujii et al. 2012). Total fungal DNA was Southern blotted and analyzed using a DIG DNA labeling and detection kit (Roche, Basel, Switzerland) to determine the fluorescence excitation of GFP and DAPI.

Fluorescence microscopy
YPyrF and YCreGFP were cultured in MM medium at 30°C for 24 h, after which the cells were collected and incubated with 1 mM 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI, Lonza, Walkersville, MD) and then analyzed under a fluorescence microscope (ZEISS, Oberkochen, Germany) to determine the fluorescence excitation of GFP and DAPI.

Other methods
Filter-paper degrading enzyme (FPase) and xylanase activities were measured as previously described (Fujii et al. 2009). The concentration of soluble protein was determined using the method of Lowry et al. (Lowry et al. 1951). The glucose concentration was determined using an HPLC system equipped with an RI-2031 Plus refractive index detector (Jasco, Tokyo, Japan) and an refractive index detector (Jasco, Tokyo, Japan) and an refractive index detector (Jasco, Tokyo, Japan) and an

Table 1 Characteristics of the A. cellulolyticus strains and plasmids used in this study

| Strain or Plasmid | Description | References |
|------------------|-------------|------------|
| Strain           |             |            |
| A. cellulolyticus Y-94 | Wild type (FERM BP-5826) | Yamanobe et al. (1987) |
| YP-4             | Uracil auxotrophic mutant derived from Y-94 | Inoue et al. (2013) |
| YDCre            | YP-4 prototrophic transformant harboring pDCre2500, creA disruptant. | This study |
| YPyrF            | YP-4 prototrophic transformant harboring a single copy pbs-pyrF in pyrF loci. | This study |
| YCreGFP          | YP-4 prototrophic transformant harboring pCreGFP | This study |
| Plasmid          |             |            |
| pbs-pyrF         | Amp' PyrF; pBluescript KS(+) derivative containing 2.7-kb fragment harboring pyrF from Y-94 | Fujii et al. (2012) |
| pDCre2500        | Amp' PyrF; pbs-pyrF derivative containing 2.5 kb upstream and 2.5 kb downstream regions of creA | This study |
| pDCre1000        | Amp' PyrF; pbs-pyrF derivative containing 1.0 kb upstream and 1.0 kb downstream regions of creA | This study |
| pCreGFP          | Amp' PyrF; pbs-pyrF derivative containing GFP and creA fused gene | This study |

Quantitative RT-PCR
Total RNA was extracted from disrupted fungal cells. Single-stranded cDNA was synthesized and then real-time quantitative PCR proceeded as described previously (Fujii et al. 2010). Table 1 lists the gene-specific primers used. The primers were designed according to the sequence of each gene in the database: endoglucanase (celSA), HV540858; endoglucanase (celSB), HV540855; cellobiohydrolase II (cel6A), AB022429; cellobiohydrolase I (cel7A), E39854; endoglucanase (cel7B), HV540856; and xylanase (xyl11B), E39857. The expression of each gene was normalized against that of the glyceraldehyde dehydrogenase gene (gpdA). Results are shown as relative expression.

Table 2 Nucleotide primers used in this study

| Primer       | Nucleotide sequence |
|--------------|---------------------|
| For plasmids construction |                   |
| creA1000u-f  | 5'-GGGTCGACTTTACGGACGTAGCTAGG-3' |
| creA1000u-r  | 5'-CCGAATTCGACGGATATTCCTCCAAC-3' |
| creA1000d-f  | 5'-CCCTTAGAGGCTGCGGCTTATAAATACCGTG-3' |
| creA1000d-r  | 5'-CGGCAGCGCATCAGTGAAGGAGAAGACT-3' |
| creA2500u-f  | 5'-GGGTGACTGACGAAAGCGAATGCGCG-3' |
| creA2500u-r  | 5'-GGGAATTCGACGAGTTGATGTTGAC-3' |
| creA2500d-f  | 5'-CCCTTAGACTTATTCACGGAACACCGAATG-3' |
| creA2500d-r  | 5'-CCGCCGGCCGCGCGCTGCCGACACCGGAT-3' |
| gfp-f        | 5'-GCGTCGACATGAGTGATGTTGA-3' |
| gfp-r        | 5'-GCGTCGACATGAGTGATGTTGA-3' |
| creAN-f      | 5'-CGAGTCGACACTTATTCACCCAGCAAGCG-3' |
| creAN-r      | 5'-CGGGATATCCGAGGCGGCGCAACAC-3' |
| For quantitative PCR |                  |
| Cel5A-f      | 5'-GGGTCGACTTTACGGACGTAGCTAGG-3' |
| Cel5A-r      | 5'-CCGAATTCGACGGATATTCCTCCAAC-3' |
| Cel5B-f      | 5'-CCGTCGACTTTACGGACGTAGCTAGG-3' |
| Cel5B-r      | 5'-CCGTCGACACTTATTCACGGAACACCG-3' |
| Cel6A-f      | 5'-GGGTCGACTTTACGGACGTAGCTAGG-3' |
| Cel6A-r      | 5'-CCGAATTCGACGGATATTCCTCCAAC-3' |
| Cel7A-f      | 5'-GGGTCGACTTTACGGACGTAGCTAGG-3' |
| Cel7A-r      | 5'-CCGAATTCGACGGATATTCCTCCAAC-3' |
| Cel7B-f      | 5'-GGGTGACTGACGAAAGCGAATGCGCG-3' |
| Cel7B-r      | 5'-GGGTGACTGACGAAAGCGAATGCGCG-3' |
| Xyl11B-f     | 5'-CTCGACTCTGACGGGTCTGGTA-3' |
| Xyl11B-r     | 5'-CTCGACTCTGACGGGTCTGGTA-3' |
| Gapdh-f      | 5'-CCGTCGACTTTACGGACGTAGCTAGG-3' |
| Gapdh-r      | 5'-CCGTCGACTTTACGGACGTAGCTAGG-3' |
Aminex HPX-87P column (Bio-Rad, Hercules, CA) fitted with a Carbo-P micro-guard cartridge (Bio-Rad). The mobile phase was double-deionized water, the flow rate was 1.0 mL/min, and the column temperature was 80°C.

### Accession numbers
The nucleotide and amino acid sequences of creA and gpdA from Y-94 are to appear in the GenBank/EMBL/DDBJ nucleotide database under accession nos. AB847424 and AB847425, respectively.

### Results

**Characterization of the putative creA gene**

First, we searched the *A. cellulolyticus* Y-94 genome database for a putative creA gene (unpublished data). A 1248 bp nucleotide sequence was found that encodes a 415 amino acid protein. A database search revealed that the amino acid sequence of this protein was similar to that of the CreA protein of *Talaromyces marneffei* (XP_002152134), *Aspergillus aculeatus* (O94166), *Aspergillus oryzae* (AK11189), and *T. reesei* (BAO9784), at 98%, 76%, 75%, and 53% identity, respectively (Figure 1). The predicted protein contains a zinc-finger domain that

![Figure 1](http://www.amb-express.com/content/3/1/73)
is conserved among the creA proteins (Figure 1). These data suggest that the nucleotide sequence we identified encodes an A. cellulolyticus Y-94 ortholog of the creA gene.

**Isolation of a creA disruptant strain**

Isolation of a strain with a disrupted creA gene is essential for analysis of the gene’s function. Gene disruption was carried out by homologous recombination, and we confirmed that creA was replaced with the pyrF marker, as shown in Figure 2. When pDCre1000 (with the homologous region length set to 1,000 bp) was introduced into YP-4, no gene-disrupted strains were isolated from the 300 transformants showing restored uracil auxotrophy (Table 3). This result was consistent with our previous finding that exogenous DNA is commonly integrated into the A. cellulolyticus genome with nonhomologous recombination (Fujii et al. 2012). On the other hand, when pDCre2500 (with the homologous region length set to 2,500 bp) was introduced, 19 gene-disrupted strains were obtained from only 71 transformants (Table 3). Southern blotting of total DNA from the YP-4 and YDCre (creA disruptant) strains revealed a 10 kb PstI DNA fragment specific for YPyrF creA but not for YDCre creA. YDCre generated a 7 kb band, indicating a deletion of the creA gene (Figure 2). These data indicate that the length of the homologous regions is important for gene disruption in A. cellulolyticus. To our knowledge, YDCre is the first A. cellulolyticus gene-disrupted strain produced by homologous recombination. We also constructed YPyrF as a control strain, which harbors a single copy of pbs-pyrF in the pyrF locus of the YP-4 genome (data not shown).

Growth of YDCre on MM and PDA medium was slower than that of YPyrF (Figure 3A), which is consistent with the growth rate of creA disruptants of other filamentous fungi. Cellular localization of CreA protein was investigated by observation of CreA-GFP fusion protein fluorescence. Figure 3B shows that fluorescence was emitted by GFP in DAPI-stained nuclei, indicating that CreA protein was localized in the nucleus. These data confirm that the cloned gene encodes CreA protein.

**The role of creA in cellulase and hemicellulase production**

YPyrF and YDCre were cultured in medium containing cellulose and glucose, and the glucose concentration in the culture supernatant was then measured. Although YPyrF consumed all of the glucose within 48 h, irrespective of the starting concentration, some glucose remained in the YDCre supernatants after 48 h of cultivation (Figure 4A).

Next, we measured the activity of the enzymes FPase and xylanase in the culture supernatant after 48 h of cultivation under various conditions. The activity in the YPyrF supernatant when the cells were grown only on cellulose (5:0) was taken as 100% (Figure 4B). The relative FPase activity in YPyrF supernatants was 142% (cellulose:glucose = 4:1), 45% (2.5:2.5), and 5% (1:4), whereas the relative xylanase activity was 155% (4:1), 101% (2.5:2.5), and 3% (1:4) (Figure 4B). When the glucose concentration was high (1:4), the FPase and xylanase activities of YPyrF were very low, indicating

![Figure 2 Disruption of the creA gene in A. cellulolyticus.](image-url)
that cellulase and xylanase production were repressed by glucose. The relative FPase activity in YDCre supernatants was 301% (5:0), 324% (4:1), 173% (2.5:2.5), and 168% (1:4), whereas the relative xylanase activity was 201% (5:0), 176% (4:1), 175% (2.5:2.5), and 85% (1:4) (Figure 4B). The activity of both enzymes was higher than that of the YPyrF enzymes under all conditions tested. Furthermore, although the activity of both enzymes in the YPyrF culture was very low at the highest glucose:cellulose ratio examined, their activity was much higher in the YDCre culture grown under the same conditions. When xylan was used as the sole carbon source, YDCre produced higher FPase and xylanase activities than YPyrF (Figure 4B). These data suggest that creA is involved in repression of cellulase and xylanase production.

We then examined FPase production by YDCre and YPyrF in time-course experiments (Figure 5). When cellulose was used as the sole carbon source, YDCre showed 1.25-fold higher FPase activity than YPyrF after 120 h of cultivation. When the strains were cultured under cellulose and glucose conditions (cellulose:glucose = 1:4), YDCre exhibited 1.3-fold higher FPase activity than YPyrF after 120 h of cultivation. Furthermore, YDCre exhibited significantly higher FPase activity than did YPyrF in the early stages of culture (after 48 h of cultivation) under both conditions, indicating that cellulase production is induced more rapidly in YDCre than in YPyrF. These data suggest that disruption of creA in A. cellulolyticus results in effective cellulase production.

| Introducing plasmids | Number of transformants | Number of creA disruptants | Gene disruption efficiency (%) |
|----------------------|-------------------------|-----------------------------|--------------------------------|
| pDCre1000            | 300                     | 0                           | 0                             |
| pDCre2500            | 71                      | 19                          | 27                            |

Figure 3 Characterization of the creA disrupted strain and the creA-GFP fused gene strain. A, Photographs showing YPyrF and YDCre cells cultured on MM and PDA media. B, YPyrF and YCreGFP were cultured in MM medium for 1 day, and then observed under a fluorescence microscope. Fluorescence emitted by GFP is indicated by an arrowhead.

Figure 4 Cellulase and xylanase activities of the strains under various culture conditions. A, Glucose consumption. The strains were cultured in cellulose- and glucose-containing medium. Initial cellulose and glucose concentrations were 10 and 40 g/L (circles), 25 and 25 g/L (triangles), and 40 and 10 g/L (squares), respectively. B, FPase and xylanase activities of the strains under various culture conditions. Initial cellulose and glucose concentrations were 50 and 0 g/L (5:0), 40 and 10 g/L (4:1), 25 and 25 g/L (2.5:2.5), and 10 and 40 g/L (1:4), respectively. Initial xylan concentration was 50 g/L. Black bars, FPase; white bars, xylanase. Data are presented as the mean of three experiments.
The role of creA in cellulase and hemicellulase gene expression

The levels of cellulase and xylanase gene expression in YD<sup>cre</sup> and YPyr<sup>F</sup> cultured for 24, 72, and 120 h were measured by quantitative RT-PCR (Figure 6A-C). Expression of cellulase and xylanase genes was observed after 24 h (e.g., relative expression ratio of <i>cel7A</i>, encoding cellubiohydrolase I, of Ypyr<sup>F</sup>; 2.0), reached their maximum levels by 72 h (18.5), and had decreased by 120 h (8.1) in both strains (Figure 6A-C). The gene <i>cel7A</i> exhibited the highest expression level among the analyzed genes, followed by <i>cel6A</i> (celllobiohydrolase II).

Figure 5 Time-dependent cellulase activity of the strains. Strains were cultured in medium containing 50 g/L of cellulose (A), or 10 g/L of cellulose and 40 g/L of glucose (B). Circles, YD<sup>cre</sup>; triangles, YPyr<sup>F</sup>. Data are presented as the mean of three experiments.

Figure 6 Expression of cellulase and xylanase genes. Strains were cultured in medium containing 50 g/L of cellulose for 24 h (A), 72 h (B), 120 h (C), or in 10 g/L of glucose and 40 g/L of cellulose for 48 h (D). Expression levels are shown relative to that of gpdA as an internal control. Black bars, YD<sup>cre</sup>; white bars, YPyr<sup>F</sup>. Data are presented as the mean of three experiments.
and cel7B (endoglucanase). Gene expression was much higher in YDCre than in YPyrF (with the exception of cel5B, endoglucanase, at 120 h), which is consistent with the result showing that cellulase and xylanase enzymatic activity are higher in YDCre than in YPyrF. These data indicate that cellulase and xylanase production in YDCre is regulated at the transcriptional level. When the strains were cultured in medium containing cellulose and glucose (culturing for 48 h; some glucose remained in the YDCre supernatants, Figure 4A), no expression was detected in YPyrF for any of the genes analyzed. In contrast, expression of all the genes was detected in YDCre (Figure 6D). These data indicate that expression of the genes analyzed in this study is repressed by glucose addition and that this repression is abolished by disruption of creA.

Discussion
In this study, we characterized the function of the A. cellulolyticus gene, creA. The deduced amino acid sequence of CreA showed that it is highly similar to CreAs produced by other filamentous fungi. In addition, CreA was found to localize in the nucleus of A. cellulolyticus. Strain YDCre showed higher cellulase and xylanase activity than strain YPyrF, and repression of enzyme activity by glucose was abolished in YDCre. These activities were regulated at the transcription level; taken together, these data suggest that CreA is a transcription factor involved in carbon catabolite repression. The present study is the first report demonstrating improved enzyme production following modification of transcriptional regulation in A. cellulolyticus.

In our previous study, we noticed that exogenous cellulose is responsive to various carbon sources. Enzyme production in a T. reesei, creA-knockout strain was shown to be higher than in the parental strain under certain conditions (Nakari-Setälä et al. 2009), which is consistent with our results described above.

In other filamentous fungi, CreA protein regulates gene expression by binding to the promoter region (Ilmen et al. 1996). Three binding sequences of CreA protein (5′-SYGGRG-3′) in other fungi were identified in a 1300 bp upstream region of cel7A of A. cellulolyticus (data not shown). Furthermore, the binding sequences were found in the upstream region of other genes analyzed in Figure 6 (data not shown). These data imply that the CreA protein of A. cellulolyticus represses gene expression by binding to the promoter regions. In T. reesei, transcription of Xyr1, which is currently considered a main inducer of cellulase and xylanase production, and of Ace1, which is a specific repressor for cellulase and hemicellulase production, were repressed by carbon catabolite repression involving CreA protein (Mach-Aigner et al. 2008; Portnoy et al. 2011). These data suggest that CreA protein of T. reesei regulates not only cellulase and hemicellulase genes but also their transcription factors. CreA protein of A. cellulolyticus may regulate other transcription factors involving cellulase and hemicellulase production as T. reesei; however, no transcription factors other than CreA of A. cellulolyticus have been investigated. Hence, further experiments, such as the identification of other transcription factors, are needed to address the mechanism of regulation of cellulase and hemicellulase production by A. cellulolyticus.

The results obtained in this study strongly indicate that disruption of creA leads to elevated cellulase and hemicellulase production in A. cellulolyticus. We are currently analyzing other transcription factors that are expected to regulate the production of these enzymes, and intend to further improve cellulase and hemicellulase production by A. cellulolyticus by modifying these factors.

Competing interests
The authors declare that they have no competing interests.

Acknowledgments
We thank Dr. Shigeki Sawayama of Kyoto University for helpful discussions. This study was supported by the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

Received: 5 December 2013 Accepted: 12 December 2013
Published: 20 December 2013

References
Aro N, Saloheimo A, Ilmen M, Penttilä M (2001) AceI, a novel transcription activator involved in regulation of cellulase and xylanase genes of Trichoderma reesei. J Biol Chem 276:24309–24314
Fujii T, Iwata K, Murakami K, Yano S, Sawayama S (2012) Isolation of uracil
Fujii T, Fang X, Inoue H, Murakami K, Sawayama S (2009) Enzymatic hydrolyzing
Inoue H, Fujii T, Yoshimi M, Taylor LE, 2nd, Decker SR, Kishishita S, Nakabayashi M,
Ilmen M, Onnela ML, Klemsdal S, Keranen S, Penttilä M (1996) Functional analysis
Goyal A, Ghosh B, Eveleigh D (1991) Characteristics of fungal cellulases. Bioresour
Kanna M, Yano S, Inoue H, Fujii T, Sawayama S (2011) Enhancement of
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with
Mach-Aigner AR, Pucher ME, Steiger MG, Bauer GE, Preis SJ, Mach RL (2008)
Lynd LR (1996) Overview and evaluation of fuel ethanol from cellulosic biomass:
Krogh KB, Mørkeberg A, Jørgensen H, Frisvad JC, Olsson L (2004) Screening
Nitta M, Furukawa T, Shida Y, Mori K, Kuhara S, Morikawa Y, Ogasawara W (2012)
Dowzer C, Kelly J (1989) Cloning of the
http://www.amb-express.com/content/3/1/73
Stricker A, Grosstessner-Hain K, Würleitner E, Mach R (2006) Xyr1 (xylanase regula-
Sehnem NT, Bittencourt LR, Camassola M, Dillon AJP (2006) Cellulase production
Portnoy T, Margeot A, Seidl-Seiboth V, Le Crom S, Ben Chaabane F, Linke R,
Wen Z, Liao W, Chen S (2005) Production of cellulase/β-glucosidase by the mixed fungi
culture Trichoderma reesi and Aspergillus phoenicis on dairy manure. Process Biochem
Yamanobe T, Mitsuishi Y, Takasaki Y (1987) Isolation of a cellulolytic enzyme
producing microorganism, culture conditions and some properties of the enzymes. Agric
Biol Chem 51:65–74
Yamanobe T, Okuda N, Oouchi K, Suzuki K (2003) This patent includes isolation of
A. cellulolyticus strain C-1, which is cellulase hyper-producing mutant strain
derived from strain Y-94. Japanese patent, Japanese patent, p 2003–135052. 13 May 2003.
doi:10.1186/2191-0855-3-73
Cite this article as: Fujii et al.: Enhancing cellulase and hemicellulase production by genetic modification of the carbon catabolite repressor gene, creA, in Acremonium cellulolyticus. AMB Express 2013 3:73.

Submit your manuscript to a SpringerOpen journal and benefit from:

► Convenient online submission
► Rigorous peer review
► Immediate publication on acceptance
► Open access: articles freely available online
► High visibility within the field
► Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com