Systematic analysis of an evolved *Thermobifida fusca* muC producing malic acid on organic and inorganic nitrogen sources

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*Thermobifida fusca* is a thermophilic actinobacterium. *T. fusca* muC obtained by adaptive evolution preferred yeast extract to ammonium sulfate for accumulating malic acid and ammonium sulfate for cell growth. We did transcriptome analysis of *T. fusca* muC on Avicel and cellobiose with addition of ammonium sulfate or yeast extract, respectively by RNAseq. The transcriptional results indicate that ammonium sulfate induced the transcriptions of the genes related to carbohydrate metabolisms significantly more than yeast extract. Importantly, *Tfu_2487*, encoding histidine-containing protein (HPr), didn’t transcribe on yeast extract at all, while it transcribed highly on ammonium sulfate. In order to understand the impact of HPr on malate production and cell growth of the muC strain, we deleted *Tfu_2487* to get a mutant strain: muCΔ2487, which had 1.33 mole/mole-glucose equivalent malate yield, much higher than that on yeast extract. We then developed an *E. coli*-*T. fusca* shuttle plasmid for over-expressing HPr in muCΔ2487, a strain without HPr background, forming the muCΔ2487S strain. The muCΔ2487S strain had a much lower malate yield but faster cell growth than the muC strain. The results of both mutant strains confirmed that HPr was the key regulatory protein for *T. fusca*’s metabolisms on nitrogen sources.

*Thermobifida fusca* is a thermophilic actinobacterium, which is an efficient degrader of plant cell walls1. *T. fusca* has been heavily studied for more than 30 years because it can produce a variety of cellulases, hemicellulases and other enzymes. These enzymes especially cellulases are thermostable and their activities retain high from pH 4 to 102–9. Previously, a mutant strain, *T. fusca* muC obtained from adaptive evolution by cultivating *T. fusca* WT on non-lethal medium, was found to accumulate malic acid from different sugars10. Malic acid is an important value-added product of the C4 diacids family, reported by the U.S. Department of Energy as the chemical of great commercial interest produced from renewable substrates11. The malic acid synthesis pathway in *T. fusca* was identified by Deng *et al.*: phosphoenolpyruvate from glycolysis pathway was converted to oxaloacetate, which was then reduced to malate12. Previously, the nitrogen sources were proved to affect the cell growth and malic acid production significantly12. In this study, we did transcriptome analysis of *T. fusca* muC on Avicel and cellobiose with addition of ammonium sulfate and yeast extract, respectively. The histidine phosphocarrier protein (Hpr or Tfu_2487) didn’t express at all in the muC strain on ammonium sulfate, with faster cell growth and less malic acid yield compared to the one on yeast extract. In order to understand the impact of HPr on the muC strain’s metabolisms, the *hpr* gene was deleted in *T. fusca* muC, forming muCΔ2487. *Tfu_2487* was then over-expressed in muCΔ2487, forming muCΔ2487S, which was grown on ammonium sulfate and yeast extract.

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Results

Effect of nitrogen sources. The muC strain was grown on four conditions with different carbon and nitrogen sources: 1) CB1: 2 g/L yeast extract, 5 g/L cellobiose; 2) CB2: 2 g/L yeast extract, 5 g/L Avicel; 3) CC1: 2 g/L ammonium sulfate, 5 g/L cellobiose; 4) CC2: 2 g/L ammonium sulfate, 5 g/L Avicel. The fermentation results are shown in Fig. 1. The specific growth rates on CC1 and CC2 were 0.218 h\(^{-1}\) and 0.221 h\(^{-1}\), which were significantly higher than those on CB1 (0.142 h\(^{-1}\)) and CB2 (0.154 h\(^{-1}\)). However, the muC strain on CB1 and CB2 had much higher malic acid yields than those on CC1 and CC2. It seems that yeast extract is much more suitable for producing malic acid, while \textit{T. fusca} muC favors ammonium sulfate to accumulate biomass.

Global gene expression and clusters of orthologous groups (COG) analysis. In order to investigate transcriptional changes on different conditions, the transcriptomes of the muC strain on CB1, CB2, CC1 and CC2 were analyzed by RNAseq. The RNAseq results were stored in the GenBank (SRA #: SRP067532). The results of transcriptomes are shown in Supplementary Table S2.

The COG distributions for the genes up-regulated and down-regulated in the transcriptomes are shown in Fig. 2. Compared to CB1, \textit{T. fusca} muC on CC1 had 357 genes up-regulated and 335 genes down-regulated; the top three categories of up-and down-regulated genes are: R: general function prediction only
Compared to CB2, *T. fusca* muC on CC2 had 292 genes up-regulated and 357 genes down-regulated. The top three categories of up-and down-regulated genes are: R: General function prediction only; C: Energy production and conversion; C: Amino acid transport and metabolism.

The observed cell growth and metabolite changes were phenotypes that largely depended on changes in carbohydrate transport and metabolism. The genes responsible for producing malic acid by muC were also mainly included in the above category. When considering genes associated with carbohydrate transport and metabolism, RNA transcripts in CC1 (as compared to the CB1 strain): 184 genes up-regulated and 27 genes down-regulated; the ones in CC2 (as compared to the CB2): 172 genes up-regulated and 10 genes down-regulated.

Because *T. fusca* muC on CC1 and CC2 used ammonium sulfate as the sole nitrogen source and *T. fusca* muC on CB1 and CB2 used yeast extract as the sole nitrogen source, the above transcriptional results indicate that ammonium sulfate induced the transcriptions of the genes related to carbohydrate metabolisms significantly more highly than yeast extract. Based on the cell growth in Fig. 1, it is indicated that the higher transcriptional levels of the genes related to carbohydrate metabolism were in agreement with the faster cell growth of the muC strain on CC1 and CC2 (ammonium sulfate as the nitrogen source).

**Genes related to cellulases.** The widely accepted mechanism for enzymatic hydrolysis of cellulose involves the synergistic activity of endocellulases, exocellulases and processive cellulases. There were 10 cellulase genes and 3 hemicellulase genes found to be highly expressed in the muC strain under all conditions (Table 1).

### Table 1. The cellulase and hemicellulase gene expression changes.

| Cellulase       | Definitions                                      | CC1-CB1 | CC2-CB2 |
|-----------------|--------------------------------------------------|---------|---------|
| Tfu_0620        | cellobiohydrolase (EC:3.2.1.91)                  | −1.08   | 1.59    |
| Tfu_0901        | endoglucanase [EC:3.2.1.4]                       | 0.75    | 1.13    |
| Tfu_0937        | beta-glucosidase [EC:3.2.1.21]                   | −1.73   | 2.79    |
| Tfu_1074        | endoglucanase [EC:3.2.1.4]                       | 0.12    | 1.78    |
| Tfu_1607        | exo-1,4-beta-glucosidase (EC:3.2.1.74)           | 0.74    | −2.17   |
| Tfu_1627        | cellulose 1,4-beta-cellobiosidase [EC:3.2.1.91]  | 0.49    | 1.61    |
| Tfu_1959        | cellulose 1,4-beta-cellobiosidase [EC:3.2.1.91]  | −0.05   | 1.82    |
| Tfu_2176        | endoglucanase [EC:3.2.1.4]                       | 1.09    | 2.12    |
| Tfu_2712        | endoglucanase [EC:3.2.1.4]                       | 0.08    | 2.38    |
| Tfu_1629        | beta-glucosidase (EC:3.2.1.21)                   | 1.36    | 1.26    |

**Hemicellulase**

| Hemicellulase   | Definitions                                      | CC1-CB1 | CC2-CB2 |
|-----------------|--------------------------------------------------|---------|---------|
| Tfu_1213        | endo-1,4-beta-xylanase [EC:3.2.1.8]              | 0.79    | 3.23    |
| Tfu_2791        | endo-1,4-beta-xylanase [EC:3.2.1.8]              | 1.39    | 3.83    |
| Tfu_2923        | endo-1,4-beta-xylanase [EC:3.2.1.8]              | −0.14   | 2.59    |

Table 1. The cellulase and hemicellulase gene expression changes. Note: LFC: log fold change. When LFC ≥ 1 or LFC ≤ −1, the changes are significantly different. CB1: 2 g/L yeast extract, 5 g/L cellobiose; CB2: 2 g/L yeast extract, 5 g/L Avicel; CC1: 2 g/L ammonium sulfate, 5 g/L cellobiose; CC2: 2 g/L ammonium sulfate, 5 g/L Avicel.

Figure 3. Cellulase activities on different conditions. CB1: 2 g/L yeast extract, 5 g/L cellobiose; CB2: 2 g/L yeast extract, 5 g/L Avicel; CC1: 2 g/L ammonium sulfate, 5 g/L cellobiose; CC2: 2 g/L ammonium sulfate, 5 g/L Avicel.

(equivalent to unclassified), E: Amino acid transport and metabolism and C: Energy production and conversion. Compared to CB2, *T. fusca* muC on CC2 had 292 genes up-regulated and 357 genes down-regulated. The top three categories of up-and down-regulated genes are: R: General function prediction only; C: Energy production and conversion; C: Amino acid transport and metabolism.

The observed cell growth and metabolite changes were phenotypes that largely depended on changes in carbohydrate transport and metabolism. The genes responsible for producing malic acid by muC were also mainly included in the above category. When considering genes associated with carbohydrate transport and metabolism, RNA transcripts in CC1 (as compared to the CB1 strain): 184 genes up-regulated and 27 genes down-regulated; the ones in CC2 (as compared to the CB2): 172 genes up-regulated and 10 genes down-regulated.

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**Genes related to cellulases.** The widely accepted mechanism for enzymatic hydrolysis of cellulose involves the synergistic activity of endocellulases, exocellulases and processive cellulases. There were 10 cellulase genes and 3 hemicellulase genes found to be highly expressed in the muC strain under all conditions (Table 1).

In Table 1, 7 out of 10 cellulase genes and 2 out of 3 hemicellulase genes of the muC strain on CC1 transcribed more than those on CB1 (LFC ≥ 0). All of the cellulase and hemicellulase genes except *Tfu_1607* of the muC strain, transcribed significantly more on CC2 than those on CB2 (LFC ≥ 1).

Although most of the cellulase gene's expressions were higher on ammonium sulfate than on yeast extract, in order to estimate the synergistic effect of the cellulases corresponding to gene expressions, the specific cellulase activities were tested. In Fig. 3, the muC strain on CC1 had much higher specific cellulase activity than the one on CB1. And the specific cellulase activity of muC on CC2 was much higher than that on CB2. Thus, the cellulase gene expressions were in agreement with the specific cellulase activity well.
yeast extract or ammonium sulfate, \( \mu C \Delta \) the \( \mu C \) strain (4.33 g/L on yeast extract with 1.10 mole-malate/mole-glucose equivalent yield). Besides, on either the one for gapdh (Tfu_2017) transcription in \( Tfu_2017 \) and \( \mu C \Delta \) replication in \( \Delta \) yield of malic acid by \( \mu C \Delta \) 2487S strain was much higher than the \( \mu C \) strain both on ammonium sulfate (0.99 g/L for \( \mu C \Delta \) of the \( \mu C \) \( \mu C \) controlled by a thiostrepton inducible promoter, tipA. The above plasmid replicated in \( \mu C \Delta \Delta \) 2487, forming the hpr 0.41 g/L DCW on yeast extract). The highest titer of malate produced by \( \mu C \Delta \) extract (0.35 g/L), however, it grew much worse than the \( \mu C \) strain (0.94 g/L DCW on ammonium sulfate and respectively. In Fig. 4, \( \mu C \Delta \) replicating plasmid, which was eliminated after the homologous recombination.

Malic acid production. The synthesis of malic acid involved phosphoenolpyruvate carboxykinase (Tfu_0083) and malate dehydrogenase (Tfu_0092). The degradation of malic acid included 'malic' enzyme (Tfu_0562), malate dehydrogenase (oxaloacetate decarboxylating) (Tfu_2390) and fumarate hydratase (Tfu_0459)\(^\text{12}\). All the genes related to malic acid metabolism were expressed highly (Supplementary Table S2). The gene expressions related to malic acid pathway were shown in Table 2.

In Fig. 1B, the \( \mu C \) strain on CB1 and CB2 produced a lot more malic acid than that on CC1 and CC2. However, the transcriptions of the major genes related to malic acid synthesis were inconsistent with the fermentation results. We further looked into the major genes related to the malic acid degradation to pyruvate, which were 'malic' enzyme (Tfu_0562) and malate dehydrogenase (oxaloacetate decarboxylating, Tfu_2390). Both genes on CC1 and CC2 transcribed significantly more highly than the one on CB1 and CB2, causing less malate accumulated by the \( \mu C \) strain on CC1 and CC2.

Table 2. The expression changes of malic acid related genes. Note: LFC: log fold change. When LFC \( \geq 1 \) or LFC \( \leq -1 \), the changes are significantly different. CB1: 2 g/L yeast extract, 5 g/L cellobiose; CB2: 2 g/L yeast extract, 5 g/L Avicel; CC1: 2 g/L ammonium sulfate, 5 g/L cellobiose; CC2: 2 g/L ammonium sulfate, 5 g/L Avicel.

| Gene   | Definition                                           | CC1-CB1 | CC2-CB2 |
|--------|------------------------------------------------------|---------|---------|
| Tfu_0092 | malate dehydrogenase                                 | −1.32   | 2.53    |
| Tfu_0562 | ‘malic’ enzyme                                       | 1.63    | 3.49    |
| Tfu_2390 | Malate dehydrogenase (Oxaloacetate decarboxylating)  | 2.04    | 2.42    |
| Tfu_0459 | Fumarate hydratase                                   | −0.58   | 1.35    |
| Tfu_2451 | Succinate dehydrogenase                              | −0.51   | 1.54    |
| Tfu_2452 | Succinate dehydrogenase                              | −0.82   | 1.37    |
| Tfu_0083 | Phosphoenolpyruvate carboxykinase                    | 2.33    | 1.54    |

PTS system related genes and the deletion of hpr (Tfu_2487). The bacterial phosphoenolpyruvate (PEP): sugar phosphotransferase system (PTS) regulates the use of carbon sources in bacteria\(^\text{14-16}\). PTS system consists of enzyme I (EI) and histidine phosphocarrier protein (HPr) and several sugar specific enzyme IIs. EI transfers phosphoryl groups from PEP to the phosphoryl carrier protein HPr. HPr then transfers the phosphoryl groups to the different transport complexes. PTSs are ubiquitous in bacteria but do not occur in archaea and eukaryotes. It has been found that EI and HPr exist while enzyme IIs are absent in \( T. fusca \)'s metabolism\(^\text{17}\). And HPr (Tfu_2487) was thought to be the regulatory protein for \( T. fusca \)'s metabolism\(^\text{17}\).

On CB1 and CB2, there was no Tfu_2487 transcription detected, while it transcribed highly on the medium on CC1 and CC2. The transcription of this gene was confirmed by RT-qPCR (Supplementary Table S1).

After searching the genome annotations of \( T. fusca \), Tfu_2765 (enzyme I) and Tfu_2487 (HPr) have been well-annotated and they both transcribed highly (Supplementary Table S2). However, EII complexes, where the carbohydrate specificity of the PTS resides, were missing in \( T. fusca \). The above results indicate that the PTS system was not complete in \( T. fusca \), but it worked for the regulatory systems to control the carbon metabolisms\(^\text{1} \).

In order to determine if the zero transcription of Tfu_2487 was the reason why the \( \mu C \) strain produced a lot more malic acid on yeast extract than ammonium sulfate, it was deleted in the \( \mu C \) strain by homologous recombination by using plasmid puC-hpr-del, forming strain muCΔ2487. The puC-hpr-del plasmid was not a replicating plasmid, which was eliminated after the homologous recombination.

The muCΔ2487 and \( \mu C \) strains were then grown on the medium with ammonium sulfate or yeast extract, respectively. In Fig. 4, muCΔ2487 grown on ammonium sulfate had a little higher biomass (0.41 g/L) than yeast extract (0.35 g/L), however, it grew much worse than the \( \mu C \) strain (0.94 g/L DCW on ammonium sulfate and 0.41 g/L DCW on yeast extract). The highest titer of malate produced by muCΔ2487 was achieved on ammonium sulfate (5.23 g/L with 1.33 mole-malate/mole-glucose equivalent yield), not on yeast extract, and it was opposite to the \( \mu C \) strain (4.33 g/L on yeast extract with 1.10 mole-malate/mole-glucose equivalent yield). Besides, on either yeast extract or ammonium sulfate, muCΔ2487 produced a lot more malic acid than the \( \mu C \) strain (Fig. 4B)\(^\text{18}\).

The above results confirm that HPr was the repressor for malic acid production.

Over expression of Tfu_2487 in muCΔ2487 strain. In order to study if the over-expression of Tfu_2487 solely could affect cell growth and malic acid production, Tfu_2487 was expressed in plasmid pYD-Tfu-hpr, a replicating plasmid designed for \( T. fusca \).

Tfu_2487 gene was cloned into the \( E. coli-T.fusca \) shuttle plasmid pYD-Tfu-4 with thiostrepton resistant gene as the selection marker. The backbone of pYD-Tfu-4 was based on pJ6021\(^\text{19}\), a replicating plasmid in Streptomyces coelicolor and puC18 in \( E. coli \). However, the replication of origin of pYD-Tfu-4 was from \( T. fusca \) to ensure the replication was in \( T. fusca \) strains. Besides, the promoter region for thiostrepton resistant gene (tsr) was replaced by the one for gapdh (Tfu_2017) transcription in \( T. fusca \) to ensure a strong transcription of tsr in \( T. fusca \). The hpr gene was cloned to the multiple cloning site of this plasmid, forming pYD-Tfu-hpr. The transcription of hpr was controlled by a thiostrepton inducible promoter, tipA. The above plasmid replicated in muCΔ2487, forming the muCΔ2487S strain. The cell growth and malic acid yield of this mutant strain was shown in Fig. 5. The biomass of the muCΔ2487S strain was much higher than the \( \mu C \) strain both on ammonium sulfate (0.99 g/L for muCΔ2487S and 0.93 g/L for \( \mu C \) strain) and yeast extract (0.86 g/L for muCΔ2487S and 0.66 g/L for \( \mu C \) strain). The yield of malic acid by muCΔ2487S (0.64 mole-malate/mole-glucose equivalent) was much lower than the \( \mu C \) and muCΔ2487 strains (0.80 and 1.33 mole-malate/mole-glucose equivalent, respectively) on ammonium sulfate.
Although yeast extract was thought to induce the production of malic acid, the malate yield of $\text{muC}^{\Delta 2487}$ (0.71 mole-malate/mole-glucose equivalent) was significantly lower than $\text{muC}$ (1.10 mole-malate/mole-glucose equivalent) and $\text{muC}^{\Delta 2487}$ strains (1.24 mole-malate/mole-glucose equivalent). The above results indicate that the over-expression of Hpr could increase the biomass, however, the malate yield was reduced significantly.

**Discussion**

*Thermobifida fusca* is a thermophilic actinobacterium. Previously, we isolated a mutant strain: *T. fusca* $\text{muC}$ from adaptive evolution. *T. fusca* $\text{muC}$ was confirmed to produce malic acid a lot more than its parent strain YX10. After identifying the malate synthesis pathway, we engineered the $\text{muC}$ strain to produce a lot more malic acid from sugars. Importantly, by fermentation optimization, the choices of nitrogen sources were found to affect the cell growth and malate production. However, the intracellular metabolisms related to the `$\text{muC}$' strain were largely unknown. In order to investigate the effect of nitrogen sources on cell growth and malate synthesis in *T. fusca* $\text{muC}$, RNAseq was employed to analyze the transcriptional changes corresponding to nitrogen sources. After analyzing the RNAseq data, the most important discovery was that $Tfu_{2487}$, annotated for histidine-containing protein (Hpr), didn't transcribe on yeast extract, however it transcribed highly on ammonium sulfate. We then deleted $Tfu_{2487}$ to get a mutant strain: $\text{muC}^{\Delta 2487}$, which produced a lot more malic acid than the $\text{muC}$ strain on ammonium sulfate (a nitrogen source bad for malate production by $\text{muC}$). However, the deletion of $Tfu_{2487}$ is not the ultimate evidence to prove the role of Hpr in cell growth and malate production. We also need to over-express it in $\text{muC}^{\Delta 2487}$, a strain without Hpr background. The $hpr$ ($Tfu_{2487}$) gene was over-expressed.
on an E. coli -T. fusca shuttle plasmid pYD-Tfu-3 in muCΔ2487, forming muCΔ2487S. The muCΔ2487S strain experienced a low malate yield and faster cell growth compared to the muC strain.

HPr is an important member in the PTS system. We analyzed the genome annotations of T. fusca, and found that the PTS system was incomplete. In bacteria, HPr transfers a phosphoryl group to the CheY domains of the sigma factor, which is then transferred to the CheA-CheB complex, resulting in a conformational change of CheA-CheB that stimulates the activity of the flagellar motor. HPr plays a crucial role in the regulation of cell growth and motility in bacteria.

Methods

Strains and cultivation. Thermobifida fusca was grown on Hagerdahl medium with addition of desired carbon and nitrogen sources. 10% V/V of pre-cultures of T. fusca were grown in the shaken flasks at 55 °C with 250 rpm rotation. The shaken flasks were sealed by the rubber stoppers to reduce the air exchange during the fermentation. 150 mL precultures were inoculated into a 5 L fermentor at 55 °C with various stirring speeds.

Molecular Work. Deletion of Tfu_2487. The general strategy of deleting Tfu_2487 in T. fusca was described previously. The brief process is: the Hpr deletion cassette included ~500 bp DNA fragment homologous to the upstream of hpr gene, the inducible promoter region of Tfu_2176 (endoglucanase) of T. fusca (genome location: 2,552,376-2,552,832, 457 bp), kanamycin resistant gene from pET-28a (+) (813 bp) and 500 bp DNA fragment homologous to the downstream of hpr gene (the sequences are shown in the supplementary file 1). The above cassette was synthesized and ligated to pUC57 by Genewiz (Suzhou, China), forming pUC-hpr-del plasmid. The resulting plasmid with right structure was then transformed to E. coli BL21 (DE3). Then, puC-hpr-del plasmid was introduced to T. fusca. The resulting plasmid was then transformed to E. coli BL21 (DE3). Then, puC-hpr-del plasmid was introduced to T. fusca.

Plasmid work. The backbone of the E. coli -T. fusca shuttle plasmid was puC18. The replication of origin of T. fusca was identified by the online tool (http://tubic.tju.edu.cn/doric/index.php)34. The DNA cassette: tfu-shuttle for T. fusca included: Kanamycin resistant gene, tsr-inducible MCS region, the gapdh promoter region of T. fusca, thiostrepton resistant gene (str) and origin of replication was synthesized by Genewiz (Suzhou, China). The puC18 was cut at NdeI and SacI and then was ligated to tfu-shuttle cassette by Gibson assembly35, which was then transformed to E. coli JM109. The transformants were picked from agar plates and subjected to colony PCR with primers hprEco-F and hprEco-R (Supplementary Table S1). The final plasmid was sequenced to verify the structure and designated as pYD-Tfu-4. The sequence and map of pYD-Tfu-4 are shown in Supplementary Figure S1.
Construction of the plasmid for expressing hpr in T. fusca. The hpr gene was amplified by primers hprF and hprR (Supplementary Table S1). The PCR product was cloned to pUCm-T vector (Sangon Biotech, Shanghai, China) and sequenced and digested by BamHI and SphI. The, the digested hpr gene was purified and ligated to pYD-Tfu-4, forming pYD-Tfu-hpr (Supplementary Figure S2).

Transformation of T. fusca and allelic exchange. The pUC-hpr-del and pYD-Tfu-4 plasmids were transformed to T. fusca protoplasts with the experimental details described previously.22 The transformants with pYD-Tfu-4 were grown on kanamycin and thiostrepton. The deletion of hpr was confirmed by diagnosis PCR using primers hprdel-F and hprdel-R (Supplementary Table S1) and directly sequenced by Sanger sequencing, thus confirming a positive recombination event.

Metabolite detection. The metabolites were detected by HPLC system (Dionex Ultimate3000) equipped with Bio-Rad HPX-87H ion exclusion column. The mobile phase was 0.005 mol/L H2SO4 at the rate of 0.6 mL/min using IR and UV detection.

RNAseq work. T. fusca muC under different conditions was grown to 24 hours and then cells were harvested by centrifugation at >10,000 g for 15 min. The cell pellets were washed by fresh medium and then spin down again to get rid of the supernatant. The above process was repeated three times. The general procedures about RNAseq work.

Statistical Analysis. In this study, the reported data were subject to log2-transformation.26 If log2(a)−log2(b) (defined as log2 fold change or LFC) ≥ 1 or ≤ −1, the difference between a and b was significant. The other statistical analysis methods were according to Deng et al.26

RT-PCR. The RT-qPCR experiments were describe previously and the primers used are shown in Supplementary Table S1.

Cellulase activity. The general process of measuring cellulase activity has been described previously.22

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**Author Contributions**

Y.D. and X.Z., conceived and designed the experiments. Y.D., J.L., Y.M. and X.Z. performed the experiments. Y.D., J.L., Y.M. and X.Z. contributed to the writing of the manuscript. All authors Y.D., J.L., Y.M. and X.Z. contributed to the analysis of data and approved the manuscript.

**Additional Information**

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