INTRODUCTION

Lignocellulosic biomass is the most abundant renewable resource on earth, and its natural degradation is a slow process. Lignocellulosic biomass can be hydrolyzed to fermentable sugars by lignocellulolytic enzymes (Binod et al., 2011; Golan, 2011) before it can be used for the production of ethanol, organic acids, and other biofuels or bio-based products (Chovau et al., 2013; Koradiya et al., 2016; Octave & Thomas, 2009; Somerville et al., 2010). However, the high costs and low hydrolysis efficiency of lignocellulolytic enzymes remain the major barriers to the

Mechanism study of novel inducer combinations containing laver powder for *Penicillium piceum* lignocellulolytic enzyme production

Wendi Jia | Mingyue Ge | Zhaokun Zhang | Dongyuan Zhang | Le Gao

Abstract

Inducers play an important role in fungus cellulase production. In this study, a combination of Avicel and laver powder, which contains cellulose, hemicellulose, other polysaccharides, and crude lipid, was used to induce lignocellulolytic enzyme production in *Penicillium piceum* H16. When Avicel was supplemented with various amounts of laver powder, cellulase activities or extracellular protein yields increased by 11.4%–102.5%, relative to those observed when Avicel alone was used. In particular, filter paper and cellobiohydrolase activities increased by as much as 39.3% and 102.5%, respectively, after supplementation with laver powder. Furthermore, laver powder supplementation enhanced the efficiency of cellulose and hemicellulose hydrolysis in crude corn stover by 39.3% and 22.2%, respectively. The results suggest that laver powder and Avicel synergistically induce lignocellulolytic enzyme production and *P. piceum* H16 growth. iTRAQ-based quantitative proteomics analysis results further demonstrate that laver powder supplementation optimized the synergistic lignocellulolytic enzyme cocktail from *P. piceum* H16 by upregulating the production of cellulase-enhancing factors and lignocellulolytic enzymes. The oligosaccharides released by laver powder may account for the synergistic induction, which may help reduce the cost of producing lignocellulolytic enzymes by filamentous fungi.

KEYWORDS

biomass conversion, induction, laver powder, lignocellulolytic enzymes, oligosaccharides, quantitative proteomics
utilization of lignocellulose for biofuel and bio-product production (Znameroski et al., 2012). Therefore, the large-scale inexpensive production of cellulase with high hydrolysis efficiency is important and beneficial to this process (Liu et al., 2016).

Most cellulases in fungi are adaptive enzymes (Waing et al., 2015) and reach full activity only in the presence of an inducer. Hence, inducers play important roles in enzyme production. Lignocellulose is a natural inducer for cellulase production. Previous studies revealed the induction efficiency of various cellulose carbon sources, including cellulose, corn stover (Juhasz et al., 2005), sorghum stover (Orencio-Trejo et al., 2016), wheat bran (Vijayaraghavan et al., 2016), rice straw (Kang et al., 2004), sugar beet pulp (Olsson et al., 2003), bagasse (Camassola & Dillon, 2007; Rocha et al., 2016), sawdust (Nwodo-Chinedu et al., 2007), switchgrass (Zhang et al., 2012), duckweed (Li et al., 2019), and soybean hulls (Ellilä et al., 2017). The cellulolytic inducer Avicel has a high cellulose content and has been recognized as the best inducer for cellulase production. Owing to its simple composition, Avicel has a specific induction effect and is limited to cellulase production. Thus, a novel inducer combination for the multilevel optimization of lignocellulolytic enzymes is urgently needed. Materials rich in polysaccharides or oligosaccharides with low lignin contents are ideal inducers that facilitate Avicel induction. However, polysaccharides or oligosaccharides, such as cellotriose, cellobiose, lactose (Dhillon et al., 2012; Fang et al., 2008; Li et al., 2017; Sehnem et al., 2006), maltose, sorbose (Nogawa et al., 2001), galactose (Karaffa et al., 2006), sophorose (Castro et al., 2014), sophorolipid (Lo & Ju, 2009), xylan (Liao et al., 2014), and other disaccharides, have extremely high costs.

Laver powder is extensively studied worldwide as medicine and food because crude polysaccharides constitute 20%–45% of its dry weight (Gong et al., 2018). Furthermore, the total amounts of cellulose and hemicellulose may constitute up to 57% of the dry weight of laver powder without lignin (Gao et al., 2015). Laver powder has excellent characteristics as an enzyme inducer. However, enzyme production with laver powder as an inducer has not been explored.

In this study, laver powder was first used as an inducer and then applied to novel inducer combinations for lignocellulytic enzyme production by *Penicillium piceum*. This paper reveals the potential induction effect of the novel inducer combinations on lignocellulolytic enzyme production, enzymatic activity, and hydrolysis efficiency. The induction mechanism of laver powder was investigated through iTRAQ-based proteomics and laver powder oligosaccharide structure analysis. This work provides a novel strategy for decreasing lignocellulytic enzyme production cost for the biomass energy industry.

## 2 MATERIALS AND METHODS

### 2.1 Fungus and laver powder

*Penicillium piceum* 9-3 was isolated from cornstalk compost obtained from Henan Province in China and preserved in the China General Microbiological Culture Collection Center (CGMCC5314). *P. piceum* 9-3 was used as the parent strain, and H16 was used as the mutant strain according to the method described by He et al. (2015). They were maintained on PDA slants at 4°C and subcultured once every 2 weeks or 1 month. Laver powder was purchased from QingDao MingYue Seaweed Group (Qingdao, China).

### 2.2 Preparation of laver powder

Laver powder samples were dried for 24 h at 65°C and then milled with a standard 60 mesh sieve. The screened material was used directly in the studies.

### 2.3 Media and fermentation

The PDA slants were washed with sterilized water, and a spore suspension with a concentration of $10^7$–$10^8$ spore/ml was prepared.

Approximately 1 ml of the spore suspension was incubated in 30 ml of preculture (glucose 10 g/L, corn steep powder 10 g/L, pH 5.0) in a 250 ml flask and cultured at 28°C and 180 rpm for 24 h. Then, 5% inoculum size of the preculture was transferred to 30 ml of production medium in a 250 ml flask and left to stand at 26°C and 180 rpm for 120 h. The production medium comprised 17 g/L glucose, 6 g/L KH$_2$PO$_4$, 1 g/L MgSO$_4$•7H$_2$O, 2.5 g/L CaCO$_3$, 2 ml/L Tween-80, and 30 g/L inducer combination and had an initial pH of 5.0. Combinations of Avicel and laver powder in ratios of 9.5:1; 9.0:1; 8.5:1, and 8.0:1 (w/w) were used. Each combination had a total amount of 30 g/L.

### 2.4 Assays for vegetative growth

The growth of vegetative mycelia induced by different inducers was measured at 24 h intervals. Conidial germination was observed on glass slides under a microscope.

### 2.5 Determination of protein concentration

Protein concentrations were determined using a trace protein concentration determination kit. The bicinchoninic acid method was used (TransGen Biotech; Gao et al., 2013).
2.6 Analysis of laver powder components and laver powder oligosaccharide

Carbohydrates in laver powder were analyzed quantitatively according to the analytical procedures of the National Renewable Energy Laboratory (Gao et al., 2015). Crude fat content was determined using the Soxhlet method, and petroleum ether was used as the solvent (Borines et al., 2013). Crude polysaccharides were determined according to the method described by Gong et al. (2018).

Laver powder was hydrolyzed with commercially purified CBHI and EGs (Megazyme, Ireland) at 30°C for 24 h. The contents of the hydrolyzates were determined with a preparative high-performance liquid phase chromatography system (Shimadzu) with a refractive index detector (Shimadzu) on an Aminex HPX-42A column (Bio-Rad) ran at a flow rate of 0.5 ml/min at 35°C. H2O was the mobile phase.

The separated laver powder oligosaccharide was degraded to monosaccharides through acidolysis. The monosaccharides were analyzed by the methods described by Gao et al. (2018).

2.7 Enzyme assay

Filter paper activity (FPA), endoglucanase activity, and β-glucosidase activity were assayed according to the method recommended by the International Union of Pure and Applied Chemistry (IUPAC; Ghose, 1987). Celllobiohydrolase was assayed with p-nitrophenol-D-cellobioside (Sigma) as the substrate (Li et al., 2019). Xylanase activity was assayed using birchwood xylan (Sigma) as the substrate (Jørgensen et al., 2005).

2.8 iTRAQ proteomics

2.8.1 iTRAQ labeling

Approximately 100 μg of extracellular proteins was collected from each sample solution for treatment. The protein samples were digested with Trypsin Gold (Promega) for 16 h at 37°C. The trypsin-to-protein ratio was 1:30. Peptides were dried through vacuum centrifugation after trypsin digestion, then reconstituted in 0.5 M TEAB and processed according to the protocol of the iTRAQ reagent kit (Applied Biosystems). The peptides were labeled with iTRAQ tags, and labeled peptide mixtures were dried through vacuum centrifugation.

2.8.2 Fractionation through strong cationic exchange

Strong cationic exchange (SCX) chromatography was performed with a LC-20AD high-performance liquid chromatography (HPLC) system (Shimadzu). The iTRAQ-labeled mixture peptides were reconstituted in buffer A (25 mM NaH2PO4 in 25% acetonitrile; pH 2.7) and loaded onto a 4.6 mm × 250 mm Ultremex SCX column containing 5 μm of particles (Phenomenex). The mixture peptides were eluted with a gradient of buffer A at a flow rate of 1.0 ml/min for 10 min; 5%–60% buffer B (1 M KCl, 25 mM NaH2PO4 in 25% ACN, pH 2.7) for 27 min; and 60%–100% buffer B for 1 min. Then, the mixture peptides were maintained in 100% buffer B for 1 min before equilibration with buffer A for 10 min and subsequent injection. The elution process was monitored by measuring absorbance at 214 nm, and fractions were collected every 1 min. The eluted peptides were pooled into 20 fractions, and each fraction was desalted with a Strata X C18 column (Phenomenex) and vacuum-dried.

2.8.3 LC-ESI-MS/MS analysis based on Triple TOF 5600

Each fraction was resuspended in buffer C (0.1% FA, 5% ACN) at a final peptide concentration of approximately 0.5 μg/μl on average and centrifuged at 20,000 × g for 10 min. The supernatants (10 μl) were individually loaded on a LC-20AD nano-HPLC (Shimadzu) with an autosampler and then onto a 2 cm C18 trap column. Then, the peptides were eluted onto a 10 cm analytical C18 column (inner diameter, 75 μm) packed in-house. The samples were loaded at 8 μl/min for 4 min. Then, a 35 min gradient was run at 300 nl/min starting from 2% to 35% buffer D (0.1% FA, 95% ACN), followed by 5 min linear gradient to 60%, linear gradient to 80% for 2 min, and 4 min maintenance at 80% B. Finally, the gradient returned to 5% in 1 min. Data acquisition was performed with a Triple TOF 5600 system (AB SCIEX, Concord, ON) fitted with a Nanospray III source (AB SCIEX) and a pulled quartz tip as the emitter (New Objectives). Data were acquired using the following conditions: ion spray voltage of 2.5 kV, curtain gas of 30 psi, nebulizer gas of 15 psi, and interface heater temperature of 150°C. The MS was operated with an RP of greater than or equal to 30,000 FWHM for the TOF MS scans. For IDA, survey scans were acquired in 250 ms, and as many as 30 product ion scans were collected after the threshold of 120 counts/s was exceeded and a charge state ranging from 2+ to 5+ was observed. Total cycle time was set at 3.3 s. Q2 transmission window was 100 Da for 100%. Four time bins were summed for each scan at a pulser frequency value of 11 kHz according to the reading of a 40 GHz multichannel time-to-digital convert detector with four anode channels for detecting ions. A sweeping collision energy of 35 ± 5 eV and iTRAQ adjust rolling collision energy were applied to all precursor ions for collision-induced dissociation. Dynamic exclusion was set for 1/2 of peak width (15 s), and the precursor was refreshed off the exclusion list.
2.9 Enzymatic hydrolysis of crude corn stover

Enzymatic hydrolysis was performed in triplicate in a 100 ml flask at 50°C for 48 h. The reaction mixtures contained 5% (w/v) crude corn stover and 20 FPU of enzyme preparations per gram substrate and had a total volume of 50 ml each. Glucose and xylose contents in the enzymatic hydrolysis liquor were measured with another HPLC system (Shimadzu, Kyoto, Japan) with a refractive index detector (Shimadzu) on an Aminex HPX-87H column (Bio-Rad) ran at a flow rate of 0.6 ml/min at 60°C. The mobile phase was 5 mM H₂SO₄ (Gao et al., 2018).

3 RESULTS

3.1 Effect of laver powder supplementation on the induction of lignocellulytic enzyme production and protein secretion

The determined components of laver powder were mainly cellulose (9.84%), hemicellulose (47.16%), crude fat (9.75%), and crude polysaccharides (33.25%). Owing to high polysaccharide and hemicellulose contents, laver powder as an inducer promoted the induction of Avicel. Novel combinations of Avicel and laver powder in ratios of 9.5:1, 9.0:1, 8.5:1, and 8.0:1 were tested. Each combination had a total amount of 30 g/L. In the mycelium growth stage, the mycelia of P. piceum were observed and compared. The mycelia of P. piceum H16 induced by an inducer combination was much thicker and longer than the mycelia of P. piceum H16 induced by Avicel alone (Figure S2). P. piceum H16 induced by the inducer combination grew faster. The novel inducer combination considerably enhanced the radial growth and growth rates of P. piceum H16. As shown in Figure 1, the FPA of P. piceum H16 was 6.7 IU/ml when Avicel only was used after 120 h of cultivation. This result is consistent with a previous result (He et al., 2015). The highest FPA of P. piceum H16 was 9.33 IU/ml, which was 39.25% higher than that of the control. This FPA was obtained when the Avicel: laver powder ratio was 8.5:1. Laver powder amounts that were higher or lower than the optimal amounts in the inducer combination decreased P. piceum H16 FPA.

Moreover, cellobiohydrolase, endoglucanase, β-glucosidase, and xylanase activities in the novel inducer combinations were markedly higher than those in the control. When the ratio between Avicel and laver powder was 8.5:1, the cellobiohydrolase, endoglucanase, β-glucosidase, and xylanase activities of P. piceum H16 increased by 102.5%, 21.16%, 68.02%, and 11.38%, respectively, and protein yield increased by 56.10%. These results suggest that laver powder has a positive effect on cellulase enzyme induction and enhances protein secretion. Changes in lignocellulolytic enzymatic activities and protein yields were similar to those in FPA.

3.2 Effect of laver powder supplementation on the hydrolysis efficiency of crude corn

For the study of the hydrolysis efficiency of lignocellulolytic enzymes induced by the novel inducer combinations, the enzymatic hydrolysis of crude corn stover was induced using the same enzyme loading of 10 FPU/g substrate. The hydrolysis efficiency of the enzyme induced by the novel inducer combination (Avicel-to-laver powder ratio of 8.5:1) increased by 39.3% and 22.2% in the cellulose and hemicellulose conversion of crude corn stover, respectively, compared with that induced by Avicel only. Changes in the hydrolysis efficiency of the lignocellulolytic enzymes were similar to the changes in FPA. These results show that the novel inducer combinations are favorable to effective lignocellulotic enzymes with balanced amounts of cellulase and hemicellulases in lignocellulolytic enzymes.

3.3 Comparative proteomic analysis of lignocellulolytic enzymes induced by Avicel alone or Avicel supplemented with laver powder

The lignocellulolytic enzyme profile was comprehensively dissected, and the reasons for the improvements in enzymatic

FIGURE 1 Hydrolysis efficiency comparison of lignocellulolytic enzymes from Penicillium piceum H16 using crude corn stover as substrate. Enzymatic hydrolysis was performed in triplicate in a 100 ml flask at 50°C for 48 h. The reaction mixtures contained 5% (w/v) crude corn stover and 20 FPU of enzyme preparations per gram substrate in a working volume of 50 ml.
activity and hydrolytic ability after the novel inducer combinations were determined through an iTRAQ-based proteomics study. The enzyme preparations from *P. piceum* 9-3 induced by Avicel, *P. piceum* H16 induced by Avicel, and *P. piceum* H16 induced by an inducer combination were concentrated and quantified using SDS-PAGE (Figure S1). iTRAQ ratios of <0.8 and >1.2 were assigned to down- and upregulation, respectively (Figures 2 and 3).

iTRAQ-based proteomics results showed that the lignocellolytic enzymes from *P. piceum* were composed of three CBHs (two CBHIs and one CBHII), nine EGs, and hemicellulase. The majority of the lignocellulosytic proteins was upregulated after a novel inducer combination was used. The GH7 and GH6 celllobiohydrolases had high iTRAQ ratios (5.645 and 8.574, respectively; Supplementary Table S1), suggesting that laver powder supplementation in the inducers improved the expression of the CBHs. The *T. reesei* genome encodes only one GH7 cellobiohydrolase, whereas *Aspergillus nidulans*, *Aspergillus fumigatus*, and *Neurospora crassa* genomes encode two GH7 celllobiohydrolases (Adav et al., 2012). *P. piceum* is an efficient cellulose degrader compared with these fungal strains possibly because it expresses unique GH proteins, including GH6 and GH7 celllobiohydrolases, which promote crystalline cellulose hydrolysis.

Of the nine endoglucanases, GH5/7 and GH61 were significantly upregulated in *P. piceum* H16 induced by the inducer combinations. Notably, GH61 enzymes constitute a unique family of copper-dependent oxidases with nonhydrolytic action. GH61 expression from *P. piceum* H16 induced by the novel combination was higher than that from *P. piceum* H16 induced by Avicel, with an iTRAQ ratio of 5.879 (Supplementary Table S1). This result suggests that laver powder supplementation can improve nonhydrolytic enzyme expression.

The conversion of cellobiose into glucose by β-glucosidase is an important regulatory process. The glucosidase (Cel3A) production yield from *P. piceum* H16 with iTRAQ ratio of 38.637 was higher than that from *P. piceum* 9-3 due to the mutations for β-glucosidase increment (He et al., 2015). Induced by the novel inducer combinations, Cel3A and Cel3B (major extracellular β-glucosidases) were upregulated with iTRAQ ratios of 4.785 and 3.365 (Supplementary Table S1). Compared with *P. piceum* 16 induced by Avicel only, consistent with the β-glucosidase activity changes. β-Glucosidases from *P. piceum* showed excellent performance in boosting cellulase yield and efficiency in our laboratory (Gao et al., 2013, 2014).

Penicillium piceum had high amounts of hemicellulases acting on the different heteropolymers of xylans and showing catalytic versatility for effective lignocellulose degradation. Notably, xylanases (GH10, 11, 30), xyloglucanase (GH74), β-xylanase (GH43), arabinofuranosidases (GH51, 54, 62), mannases (GH2,5, 47,76), pectate lyase (PL1,20) and feruloyl esterases (CE1) were significantly upregulated. Xyloglucanase promotes substrate degradation by removing the hemicellulose– lignin barrier (Song et al., 2016). Feruloyl esterases can facilitate cellulose hydrolysis by hydrolyzing ester bonds that cross-link lignin and xylan (Song et al., 2016). However, feruloyl esterases from *P. piceum* H16 were downregulated after mutation from *P. piceum* 9-3. The novel inducer combinations successfully adjusted and enhanced feruloyl esterase expression. Some hemicellulases, such as α-glucosidase (GH71), exo-β-1,3-glucanase (GH55), amylase (GH13), glucoamylase (GH15), chitinase (GH18), and galactosidase (GH27, 36), were downregulated and had uncharacterized functions in lignocellulose degradation. The large amount of galactose released from laver powder hydrolyzed by the lignocellulytic enzymes showed an inhibitory effect on galactosidase expression. Collectively, the iTRAQ protein quantitation data reveal that the novel inducer combinations can optimize enzyme systems by upregulating all

![Figure 2](https://example.com/figure2.png)  
**Figure 2** Hierarchical clustering of lignocellulytic enzymes from *Penicillium piceum* 9-3 induced by Avicel, *P. piceum* H16 induced by Avicel, and *P. piceum* 9-3 induced by the novel inducer combinations. Approximately 100 μg of extracellular proteins were collected and digested with Trypsin Gold for 16 h at 37°C. The peptides were labeled with iTRAQ tags. Then, the labeled peptide mixtures were dried through vacuum centrifugation and analyzed with LC-ESI-MS/MS. iTRAQ ratios of <0.8 and >1.2 were assigned for down- and upregulation, respectively. Upregulated protein expression levels are displayed in red, the downregulated expression levels are in green, and the intermediate values are in shades of red and green.
3.4 | Potential mechanisms underlying the synergistic induction of lignocellulytic enzyme production by Avicel and laver powder supplementation

Laver powder was hydrolyzed using commercial CBHIs and EGs, which were constitutively expressed or expressed at the early stages of fungal growth on lignocellulose. HPLC analysis of the hydrolysis products shows that the polymerization degree of oligosaccharides released from laver powder ranged from 2 to 7. Pentose content in the laver powder oligosaccharides was the highest, whereas the concentrations of disaccharides and trisaccharides, which are generally considered the inducers of lignocellulytic enzymes, were relatively low (Fritscher et al., 1990; Znameroski et al., 2012). The monosaccharides derived from the hydrolysis of laver powder polysaccharides were mainly galactose, mannose, glucose, and 3,6-anhydro-galactose. Further analysis suggests that the molar ratio of galactose and glucose in disaccharides was approximately 1:1, and the structure of the major disaccharide was speculated to be galactose (β-1,4) glucose. Meanwhile, the molar ratio between galactose and 3,6-anhydro-galactose was approximately 2:1 in the trisaccharides, and the structure of the major trisaccharide was speculated to be galactose (β-1,4) 3,6-anhydro-galactose (β-1,3) galactose. Therefore, galactose was considered a major residue in laver powder polysaccharide chains, as in a previous report (Gong et al., 2018). For the first time, laver powder oligosaccharide with galactose as the main residue was demonstrated to have strong inducing effects on cellulase and hemicellulase production.

4 | DISCUSSION

Laver powder was first used as an inducer and then applied to novel inducer combinations for the lignocellulytic enzyme production of P. piceum H16. When Avicel was supplemented with various amounts of laver powder, cellulase activities or extracellular protein yields increased by 11.4%–102.5%, relative to those observed when Avicel alone was used. In particular, filter paper and cellobiohydrolase activities increased by as much as 39.3% and 102.5%, respectively, after supplementation with laver powder. Furthermore, laver powder supplementation enhanced the efficiency of cellulose and hemicellulose hydrolysis in crude corn stover by 39.3% and 22.2%, respectively. These results showed that the novel inducer combinations are favorable to effective lignocellulotic enzymes with balanced amounts of cellulase and hemicellulases in lignocellulytic enzymes. A previous study showed that the hydrolysis rate of cellulase in Trichoderma reesei increased by 28% by using steam-exploded corn stover as the substrate and inducing enzymes with 50 g/L duckweed (Li et al., 2019). Moringa straw induction improved β-glucosidase activity 6.41-fold (Vazquez-Montoya et al., 2020), which was originally low according to the IUPAC method. Compared with previous, laver powder showed a more apparent inducible effect.

iTRAQ-based proteomics results the novel inducer combinations optimized lignocellulolytic enzyme systems and improved hydrolytic efficiency by upregulating lignocellulolytic enzymes and cellulase-enhancing factors. Compared
with *P. piceum* 16 induced by Avicel only, the changes of upregulated lignocellulolytic enzymes consistent with the enzymatic activity changes. The upregulated cellulase such as β-glucosidases and cellobiohydrolase from *P. piceum* showed excellent performance in boosting cellulase yield and efficiency in our laboratory (Gao et al., 2013, 2014). The addition of GH61 as cellulase-enhancing factors can significantly boost the hydrolytic efficiency of cellulase mixtures (Beeson et al., 2012; Quinlan et al., 2011). The hydrolytic efficiency of lignocellulolytic enzymes induced by the novel inducer combination may be related to the upregulation of cellulase-enhancing factors.

Galactose was identified a major residue in the oligosaccharides released from laver powder. To examine whether the synergistic induction for lignocellulolytic enzyme production is related to galactose, we replaced laver powder supplementation with an equal amount of D-galactose. In the initial stage of the enzyme production of *P. piceum*, D-galactose supplementation improved cellulase production rate. However, the induction effect of galactose was not obvious during cellulase accumulation (Figure S3). Compared with laver powder, D-galactose showed little effect on cellulase enzyme induction and protein secretion enhancement in *P. piceum* (Table 1). Our results corroborate the previous finding that D-galactose can act as an inducer at slow growth rates (Karaffa et al., 2006). Laver powder supplementation can apparently increase enzymatic activities and hydrolysis efficiency. Therefore, the oligosaccharides released by laver powder may be real inducers. The structures of disaccharides and trisaccharides with galactose as the main residue are similar to those of cellobiose and cellotriose. Cello-oligosaccharides are generally considered good inducers for cellulase production. In this study, the induction of laver powder oligosaccharide was found to be better than that of cello-oligosaccharides, and thus laver powder oligosaccharide was considered a good substitute for cello-oligosaccharides. For the first time, laver powder oligosaccharide with galactose as the main residue was found to be the real inducer. It exhibited better inducing effects on lignocellulolytic enzyme production than cello-oligosaccharides. Thus, laver powder is a potential lignocellulosic material for the industrial production of lignocellulolytic enzymes.

**ACKNOWLEDGEMENTS**

This work was supported by the National Key Research and Development Program of China (2018YFE0107100, 2018YFA0902200), Tianjin Synthetic Biotechnology Innovation Capacity Improvement Project (Grant No. TSBICIP-KJGG-006), Tianjin Science and Technology Support Program Project (18ZXNYENC00150), Jilin Province, Chinese Academy of Sciences and Science and Technology Cooperation High-tech Industrialization Fund Project (2019SYHZ0012).

**CONFLICT OF INTEREST**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**ORCID**

Le Gao https://orcid.org/0000-0003-3916-5032

**REFERENCES**

Adav, S. S., Chao, L. T., & Sze, S. K. (2012). Quantitative secretomic analysis of *Trichoderma reesei* strains reveals enzymatic composition for lignocellulosic biomass degradation. *Molecular & Cellular Proteomics, 11*(7), 15.
Beeson, W. T., Phillips, C. M., Cate, J. H. D., & Marietta, M. A. (2012). Oxidative cleavage of cellulose by fungal copper-dependent polysaccharide monoxygenases. *Journal of the American Chemical Society*, 134(2), 890–892.

Binod, P., Janu, K. U., Sindhu, R., & Pandey, A. (2011). Hydrolysis of lignocellulosic biomass for bioethanol production. In A. Pandey, C. Larroche, S. C. Ricke, C.-G. Dussap, & E. Gnansounou (Eds.), *Biofuels* (pp. 229–250). Academic Press.

Borines, M. G., de Leon, R. L., & Cuello, J. L. (2013). Bioethanol production from the macroalgae Sargassum spp. *Bioresource Technology*, 138, 22–29.

Camassola, M., & Dillon, A. J. (2007). Production of cellulases and hemicellulases by *Penicillium echinulatum* grown on pretreated sugar cane bagasse and wheat bran in solid-state fermentation. *Journal of Applied Microbiology*, 103, 2196–2204.

Castro, L. D. S., Pedersoli, W. R., Antonio, A. C. C., Steinordoff, A. S., Silva-Rocha, R., Martinez-Rossi, N. M., Rossi, A., Brown, N. A., Goldman, G. H., Faa, V. M., Persinoti, G. F., & Silva, R. N. (2014). Comparative metabolism of cellulose, sophorose and glucose in *Trichoderma reesei* using high-throughput genomic and proteomic analyses. *Biotechnology for Biofuels*, 7, 17.

Chovau, S., Degrauwe, D., & Van der Bruggen, B. (2013). Critical analysis of techno-economic estimates for the production cost of lignocellulosic bio-ethanol. *Renewable & Sustainable Energy Reviews*, 26, 307–321.

Dhillon, G. S., Brar, S. K., Kaur, S., Metahni, S., & M’Hamdi, N. (2012). Lactosin as a moistening medium and crude inducer for fungal cellulase and hemicellulase induction through solid-state fermentation of apple pomace. *Biomass & Bioenergy*, 41, 165–174.

Ellila, S., Fonseca, L., Uchima, C., Cota, J., Goldman, G. H., Saloheimo, M., Sacon, V., & Siika-aho, M. (2017). Development of a low-cost cellulase production process using *Trichoderma reesei* for Brazilian biorefineries. *Biotechnology for Biofuels*, 10, 17.

Fang, X., Yano, S., Inoue, H., & Sawayama, S. (2008). Lactose enhances cellulase production by the filamentous fungus *Acremonium cellulolyticus*. *Journal of Bioscience and Bioengineering*, 106(2), 115–120.

Fritschel, C., Messner, R., & Kubicek, C. P. (1990). Cellulbiose metabolism and cellubiohydrolase I biosynthesis in *Trichoderma reesei*. *Experimental Mycology*, 14, 451–461.

Gao, L., Chen, S. L., & Zhang, D. Y. (2018). Neural network prediction of corn stover saccharification based on its structural features. *Biomed Research International*, 1–7.

Gao, L., Gao, F., Jiang, X., Zhang, C., Zhang, D., Wang, L., Wu, G., & Chen, S. (2014). Biochemical characterisation of a new β-glucosidase (Cel3E) from *Penicillium piceum* and its application in boosting lignocelluloses bioconversion and forming disaccharide inducers: New insights into the role of β-glucosidase. *Process Biochemistry*, 49, 768–774.

Gao, L., Gao, F., Zhang, D. Y., Zhang, C., Wu, G. H., & Chen, S. L. (2013). Purification and characterization of a new beta-glucosidase from *Penicillium piceum* and its application in enzymatic degrad-ation of delignified corn stover. *Bioresource Technology*, 147, 658–661.

Gao, L., Li, D. M., Gao, F., Liu, Z. Y., Hou, Y. Y., Chen, S. L., & Zhang, D. Y. (2015). Hydroxyl radical-aided thermal pretreatment of algal biomass for enhanced biodegradability. *Bioresource Technology*, 8, 11.

Ghose, T. K. (1987). Measurement of cellulase activities. *Pure and Applied Chemistry*, 59, 257–268.

Golan, A. E. (2011). Cellulase: Types and action, mechanism, and uses. Nova Science Publishers.

Gong, G. P., Zhao, J. X., Wang, C. J., Wei, M., Dang, T. T., Deng, Y. N., Sun, J., Song, S., Huang, L. J., & Wang, Z. F. (2018). Structural characterization and antioxidant activities of the degradation products from Laver powder-haitenansis polysaccharides. *Process Biochemistry*, 74, 185–193.

He, R. L., Cai, P. L., Wu, G. H., Zhang, C., Zhang, D. Y., & Chen, S. L. (2015). Mutagenesis and evaluation of cellulase properties and cellulose hydrolysis of *Talaromyces piceus*. *World Journal of Microbiology & Biotechnology*, 31(11), 1811–1819.

Jorgensen, H., Morkeberg, Å., Krogh, K. B. R., & Olsson, L. (2005). Production of cellulases and hemicellulases by three *Penicillium* species: Effect of substrate and evaluation of cellulase adsorption by capillary electrophoresis. *Enzyme and Microbial Technology*, 36(1), 42–48.

Juhasz, T., Szengyel, Z., Reczey, K., Siika-Aho, M., & Viikari, L. (2005). Characterization of cellulases and hemicellulases produced by *Trichoderma reesei* on various carbon sources. *Process Biochemistry*, 40(11), 3519–3525.

Kang, S. W., Park, Y. S., Lee, J. S., Hong, S. I., & Kim, S. W. (2004). Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. *Bioresource Technology*, 91, 153–156.

Karaffa, L., Fekete, E., Gamauf, C., Szentirmai, A., Kubicek, C. P., & Seibith, B. (2006). D-Galactose induces cellulase gene expression in *Hypocrea jecorina* at low growth rates. *Microbiology*, 152(5), 1507–1514.

Koradiya, M., Duggirala, S., Tipple, D., & Dave, S. (2016). Pretreatment optimization of Sorghum pioneer biomass for bioethanol production and its scale-up. *Bioresource Technology*, 199, 142–147.

Li, C., Li, D. M., Feng, J., Fan, X., Chen, S. L., Zhang, D. Y., & He, R. L. (2019). Duckweed (*Lemma minor*) is a novel natural inducer of cellulase production in *Trichoderma reesei*. *Journal of Bioscience and Bioengineering*, 127(4), 486–491.

Li, C. C., Lin, F. M., Zhou, L., Qin, L., Li, B. Z., Zhou, Z. H., Jin, M. J., & Chen, Z. (2017). Cellulase hyper-production by *Trichoderma reesei* mutant SEU-7 on lactose. *Biotechnology for Biofuels*, 10, 15.

Liao, H. P., Li, S. X., Wei, Z., Shen, Q. R., & Xu, Y. C. (2014). Insights into high-efficiency lignocellulolytic enzyme production by *Penicillium oxalicum* GZ-2 induced by a complex substrate. *Biotechnology for Biofuels*, 7, 17.

Liu, G., Zhang, J., & Bao, J. (2016). Cost evaluation of cellulase enzyme for industrial-scale cellulose ethanol production based on rigorous Aspen Plus modeling. *Bioprocess and Biosystems Engineering*, 39(1), 133–140.

Lo, C. M., & Ju, L. K. (2009). Sophorolipids-induced cellulase production in cocultures of *Hypocrea jecorina* Rut C30 and Candida bombicola. *Enzyme and Microbial Technology*, 44(2), 107–111.

Nogawa, M., Goto, M., Okada, H., & Morikawa, Y. (2001). L-Sorbose induces cellulase gene transcription in the cellulolytic fungus *Trichoderma reesei*. *Current Genetics*, 38(6), 329–334.

Nwodo-Chinedu, S., Okochi, V. I., Smith, H. A., Okafor, U. A., Onyegeme-Okerenta, B. M., & Omidiji, O. (2007). Effect of carbon sources on cellulase (EC 3. 2. 1. 4) production by *Penicillium chrysogenum* PCLS01. *African Journal of Biochemistry Research*, 1, 6–11.

Octave, S., & Thomas, D. (2009). Biorefinery: Toward an industrial metabolism. *Biochimie*, 91(6), 659–664.
Olsson, L., Christensen, T., Hansen, K. P., & Palmqvist, E. A. (2003). Influence of the carbon source on production of cellulases, hemicellulases and pectinases by *Trichoderma reesei* Rut C-30. *Enzyme and Microbial Technology, 33*(5), 612–619.

Orencio-Trejo, M., Torres-Granados, J., Rangel-Lara, A., Beltrán-Guerrero, E., García-Aguilar, S., Moss-Acosta, C., Valenzuela-Soto, H., De la Torre-Zavala, S., Gastelum-Arellanez, A., Martínez, A., Tiessen, A., Díaz-Mireles, E., & Lozoya-Gloria, E. (2016). Cellulase and xylanase production by the mexican strain *Talaromyces stollii* LV186 and its application in the saccharification of pretreated corn and sorghum stover. *BioEnergy Research*, 9(4), 1034–1045.

Quinlan, R. J., Sweeney, M. D., Lo Leggio, L., Otten, H., Poulsen, J. C. N., Johansen, K. S., Krogh, K., Jørgensen, C. I., Tovborg, M., Anthonsen, A., Tryfona, T., Walter, C. P., Dupree, P., Xu, F., Davies, G. J., & Walton, F. H. (2011). Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components. *Proceedings of the National Academy of Sciences of the United States of America, 108*(37), 15079–15084.

Rocha, V. A. L., Maeda, R. N., Pereira, N., Kern, M. F., Elias, L., Simister, R., Steele-King, C., Gomez, L. D., & McQueen-Mason, S. J. (2016). Characterization of the cellulolytic secretome of *Trichoderma harzianum* during growth on sugarcane bagasse and analysis of the activity boosting effects of swollenin. *Biotechnology Progress, 32*(2), 327–336.

Sehnem, N. T., De Bittencourt, L. R., Camassola, M., & Dillon, A. J. P. (2006). Cellulase production by *Penicillium echinulatum* on lactose. *Applied Microbiology and Biotechnology, 72*(1), 163–167.

Somerville, C., Youngs, H., Taylor, C., Davis, S. C., & Long, S. P. (2010). Feedstocks for lignocellulosic biofuels. *Science, 329*, 790–792.

Song, W. X., Han, X. L., Qian, Y. C., Liu, G. D., Yao, G. S., Zhong, Y. H., & Qu, Y. B. (2016). Proteomic analysis of the biomass hydrolytic potentials of *Penicillium oxalicum* lignocellulolytic enzyme system. *Biotechnology for Biofuels, 9*, 15.

Suzuki, H., Igarashi, K., & Samejima, M. (2010). Cellotriose and cellotetraose as inducers of the genes encoding cellobiohydrolases in the basidiomycete *phanerochaete chrysosporium*. *Applied and Environmental Microbiology, 76*(18), 6164–6170.

Vazquez-Montoya, E. L., Castro-Ochoa, L. D., Maldonado-Mendoza, I. E., Luna-Suarez, S., & Castro-Martinez, C. (2020). Moringa straw as cellulase production inducer and cellulolytic fungi source. *Revista Argentina De Microbiologia, 52*(1), 4–12.

Vijayaraghavan, P., Prakash Vincent, S. G., & Dhillon, G. S. (2016). Solid-substrate bioprocessing of cow dung for the production of carboxymethyl cellulase by *Bacillus halodurans* IND18. *Waste Management, 48*, 513–520.

Waing, K. G. D., Gutierrez, J. M., Galvez, C. T., & Undan, J. R. (2015). Molecular identification of leaf litter fungi potential for cellulose degradation. *Mycosphere, 6*(2), 139–144.

Zhang, L., Liu, Y., Niu, X., Liu, Y., & Liao, W. (2012). Effects of acid and alkali treated lignocellulosic materials on cellulase/xylanase production by *Trichoderma reesei* Rut C-30 and corresponding enzymatic hydrolysis. *Biomass and Bioenergy, 37*, 16–24.

Znameroski, E. A., Coradetti, S. T., Roche, C. M., Tsai, J. C., Iavarone, A. T., Cate, J. H. D., & Glass, N. L. (2012). Induction of lignocellulose-degrading enzymes in *Neurospora crassa* by cello-dextrins. *Proceedings of the National Academy of Sciences of the United States of America, 109*(16), 6012–6017.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Jia W, Ge M, Zhang Z, Zhang D, Gao L. Mechanism study of novel inducer combinations containing laver powder for *Penicillium piceum* lignocellulolytic enzyme production. *GCB Bioenergy*. 2021;13:656–664. https://doi.org/10.1111/gcbb.12811