

## Research Progress of Lignocellulase of Filamentous Fungi

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### Abstract

Since the beginning of the 21st century, the petrochemical energy crisis has become increasingly prominent. At the same time, the use of oil has also brought a series of environmental problems. It is urgent to find new sustainable green energy. Biomass is the largest renewable resource in nature. Using lignocellulosic biomass to produce degradable products to replace part of oil is an important guarantee for human sustainable development. Lignocellulose is degraded into fermentable sugars by enzymes, which plays a key role in the process of biotransformation. Filamentous fungi are important sources of lignocellulose degrading enzymes, and can secrete cellulase efficiently. Therefore, improving the efficiency of cellulase production by filamentous fungi is conducive to the industrial application of cellulase producing strains. At present, the rational transformation of strains by genetic engineering is of great significance to improve the enzyme production efficiency of filamentous fungi.

### Keywords

Filamentous Fungi; Cellulase; *Penicillium Oxalicum*.

### 1. Introduction

In today's world, the excessive consumption of oil resources, looking for green, clean and sustainable new energy is imminent, it is imperative to speed up the transformation of new and old kinetic energy. Biomass resources have the advantages of rich raw materials and renewable, which has been widely concerned [1]. Using biomass to produce liquid fuel ethanol instead of petroleum is an important way to speed up high-quality development. The production of liquid fuel ethanol from corn, sweet potato and other food resources has great limitations both now and in the future. The production of liquid fuel ethanol from non grain biomass lignocellulose not only has the characteristics of clean and environmental protection, but also does not compete with human beings for food resources [2]. Agricultural waste is one of the largest biomass resources in the world with low cost and large quantity, which is the best resource for the production of second-generation fuel ethanol. Refining fuel ethanol from biomass provides a new way to solve rural agricultural waste economically and environmentally, which is a great plan for the country and the people. Lignocellulose has a complex structure, which is difficult to be degraded. Among all kinds of biomass conversion methods, biological degradation of lignocellulose is a green and mild conversion method [3]. At present, cellulase produced by fungi is the main enzyme used in biomass lignocellulose conversion [4]. However, the cost of fungal enzyme production is high, and the profit of fermentable sugar obtained by degradation is low, which seriously restricts the market of lignocellulose enzyme. The low yield and hydrolysis efficiency of fungal cellulase make fungal cellulase lack of competitiveness in large-scale industrial production [5]. The effective way to solve this problem is to transform the high-yield strains, and improve the enzyme yield and hydrolysis efficiency mainly from three aspects: first, the gene directed transformation of strains; Second, the transformation of enzyme system; The third is the optimization of fermentation conditions. Among them, the most effective one is to use the molecular technology of gene editing to carry out genetic transformation of strains, which is of great significance to realize the industrial production of fungal cellulase.

## 2. Major cellulase producing fungi

Many fungi in nature have the ability to degrade lignocellulose. They decompose the carbon source in the living environment into energy that can provide energy for their own growth and metabolism [6]. Cellulase producing fungi have natural ability to produce enzymes, and can secrete complex cellulase systems to cope with the changing environment of lignocellulose. In the long-term evolution process, fungi have formed their own unique mechanism of cellulase synthesis [7]. The research on lignocellulose degradation by natural fungi has a history of nearly one hundred years. There are many enzyme producing fungi in nature. At present, the most studied enzyme producing fungi are *Trichoderma reesei*, *Neurospora crassa*, *Penicillium oxalicum*, These fungi have similar regulatory mechanisms and self regulatory characteristics in the expression and synthesis of lignocellulose, and there are some differences in cellulase genes among different fungi. In recent years, it has become an effective way to understand enzyme producing strains by means of genetic engineering, and many strains have completed genome sequencing. Through the study of the main enzyme producing fungi, we can find the homologous regulatory factors in different strains, and learn more about the enzyme producing mechanism of filamentous fungi, so as to realize the transformation of high-yield strains in different fungi.

### 2.1 *T reesei*

*T reesei*, a filamentous fungus, has a long history of research. The strain has a strong ability to secrete cellulase, and it is also the first enzyme producing fungus to complete the whole genome sequencing [8]. It opened the prelude of studying cellulase regulation of filamentous fungi from gene level. At present, the high-yield strains of *T reesei* have been put into production in enzyme preparation companies at home and abroad. *T reesei* has strong ability to degrade cellulose, especially crystalline cellulose [9]. However, *T reesei* has some shortcomings in the composition of cellulase system. The activity of glucosidase was insufficient. As an important part of cellulase system, glucosidase is responsible for the decomposition of cellobiose to glucose and the final enzymolysis of ring segments. *T reesei*. The lack of glucosidase activity limits its ability to hydrolyze more complex lignocellulosic materials. Moreover, the number of genes encoding cellulase in *T reesei* genome is small, and the number of targets for genetic modification is limited. However, the High Cellulase Producing Strain obtained by *T reesei* mutation is still the strain with the strongest industrial cellulase production ability, and the regulation network of cellulase expression of *T reesei* still needs further study.

### 2.2 *N crassa*

The filamentous fungi, which grow in the global tropics and subtropics, can produce cellulase rapidly and produce relatively complete cellulase system. There are long-term studies on the rough vein fungus. The whole genome sequencing of the strain has been completed. The results show that the genome contains a large number of cellulase genes, hemicellulase genes and transcription factors [10] This genetic background makes the rough vein spore a perfect strain to study the mechanism of cellulase gene expression regulation. In addition, the whole genome knockout library was constructed by the strain of the strain, which provided a strong support for the study of the transcriptional regulation of cellulase gene, and also provided a template for other enzyme producing fungi. At present, there are many studies on the enzyme production mechanism of the rough vein spore, for example, the regulatory factor *clr-2* is found in the rough vein spore [11]. After overexpression of the regulatory factor, the cellulase activity of mutant increased significantly [12]. After the knockout of the cellulase gene in the rough vein spore, the growth of the strain on cellulose medium was inhibited [13].

### 2.3 *P oxalicum*

*P oxalicum*, a kind of aerobic filamentous fungus, grows faster than other filamentous fungi [14]. The secretion of enzyme is rich, and the proportion of enzyme components is reasonable,  $\beta$ - Glucosidase has higher enzyme activity [15]. *P oxalicum* high-yield cellulase strains have been applied to the industrial production of cellulase, but also in the industrial production except *Trichoderma*. It has

been applied in food industry and feed processing industry [16]. *P. oxalicum* is a strain produce cellulase relatively high. For *P. oxalicum*, researchers at home and abroad have carried out extensive research from various histochemistry. At present, *P. oxalicum* has completed the whole genome sequencing, identified most of the cellulase genes, and annotated about 10000 protein functions, including the main transporters, transcription factors, signal transduction proteins and other related proteins in penicillin oxalate group, The annotation of protein function provides the basis for screening the cellulase regulatory factors of *P. oxalicum*. [17-20]

### 3. Cellulase

The main cellulase system was composed of three cellulases secreted from fungi outside the cell. Endo, respectively-  $\beta$ - 1, 4-glucose, eg, cellobiohydrolase (CBH) and  $\beta$ - Glucosidase. All three cellulases belong to glycoside hydrolases, and the three cellulases jointly complete the degradation process of cellulose.

The role of endoglucanase is to act on cellulose from the inside of the cellulose chain, cut the long chain cellulose short, and produce the short chain cellulose containing the end of the reductive reaction.

Exoglucanase is a kind of fiber-based glucose or oligosaccharide which is cut from the end of the cellulose chain. It is also called fibrodextranase. The exoglucanase can be divided into two types: CBH I and CBH II. CBH I degrades cellulose from the reduction end and CBH II from the non reducing end.

$\beta$ - Glucosidase is mainly used in the fiber glucose after the cut-off cellulase, which can degrade the fiber glucose,  $\beta$ - Glucosidase, as a part of cellulase system, plays a role in enzymatic hydrolysis of cellulose completely. Although it does not directly affect cellulose, it is not directly affected by glucosidase  $\beta$ - Glucosidase is an indispensable part of the cellulase system of filamentous fungi. The main enzyme producing filamentous fungi contain  $\beta$ - Glucosidase gene, including 3 in Trichoderma Richter genome  $\beta$ - Glucosidase gene, which contains 11 genes in *P. oxalicum* genome  $\beta$ - Glucosidase gene, including 6 intracellular  $\beta$ - Glucosidase gene and 5 extracellular  $\beta$ - Glucosidase gene. As an important part of cellulase system,  $\beta$ - Glucosidase has been studied in a large number of molecular levels. In addition to *bg12* gene in *P. oxalicum*, the cellulase activity of *P. oxalicum* has been improved. Studies have shown that,  $\beta$ - Glucosidase is involved in the induction of cellulase gene in fungi in addition to the hydrolysis of fibrodisease.

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