Application of Aspergillus Niger in the resourcable utilization of straw

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Abstract. As its large quantity, complex structure and composition, crop straw needs a variety of enzymes for synergistic action in the process of resourcable utilization. Various enzymes produced by Aspergillus Niger, such as cellulase, hemicellulase, lignin enzyme and pectinase, can degrade straw components and play an important role in straw resource recovery. In this paper, the advantages of Aspergillus Niger in straw degradation were reviewed, the research progress of enzymes produced by Aspergillus Niger using straw was introduced, and the further research direction of Aspergillus Niger was also prospected.

1. Introduction

China is known as a large agricultural country with abundant biomass resources. Crop straw is an important biological resource in the crop production system, which is also the fourth largest energy source of the world after coal, oil and natural gas. At present, more than 2 billion tons of straw are produced all over the world each year, and the annual output of various crop straws in China has reached about 900 million tons by 2016 [1], ranking first in the world. The comprehensive utilization of straw in China still has problems resulting in the waste of straw resources, such as the relatively extensive level, narrow utilization range, incomplete comprehensive utilization policy, incineration, abandonment and great loss [2]. It is a difficult problem of modern agriculture to make full use of these resources without environment pollution. Physical methods and chemical methods have been commonly used with many shortcomings, such as the pollution during straw processing, the decrease of nutritional value, and the unsuitability for large-scale promotion. The microbial method has many advantages in straw conversion with high nutritional value, short cycle, renewable, etc., which can improve the comprehensive benefits of straw utilization. As the adaptability and versatility of microorganisms to transform straw, the microbial treatment of straw is considered the most promising treatment method.

As its large amount, complex structure and components, straw needs synergistic reactions of a variety of enzymes for its resourcable utilization. Filamentous fungi, especially Aspergillus Niger, can produce cellulase, hemicellulase, lignase, pectinase and other enzymes that hydrolyze straw, which plays an important role in straw resourcable utilization. Moreover, Aspergillus Niger is a model microorganism expressing enzymes for industrial use. Many enzymes have been successfully expressed in Aspergillus Niger, such as lignin peroxidase, ferulic acid esterase and manganese peroxidase. Therefore, the
advantages of *Aspergillus Niger* in degrading straw are in summarized this paper to provided reference to its application in straw resource utilization.

2. Advantages of *Aspergillus Niger* in straw degradation

2.1. Rich in straw degradation enzymes

The main components of straw are cellulose, hemicellulose, lignin and pectin. Cellulose degrading enzymes include endoglucanase, exoglucanase and β-glucosidase. Enzymes for hemicellulosic degradation include β-xylanase, β-xylosidase, endo-β-mannanase, β-mannosidase, α-galactosidase, α-arabinofuranase, α-glucosidase, acetylxylanesterase, ferulic esterase and p-coumaresterase. Ligninolysis enzymes include lignin peroxidase, laccase, manganese peroxidase and a versatile peroxidase. Pectin degrading enzymes include pectin cleavage synthase, polygalacturonase and invertase [3].

Filamentous fungi, such as *Aspergillus Niger*, *Aspergillus oryzae*, *Rhizopus oryzae*, and *Trichoderma reesei*, are important sources of straw hydrolases [4]. Among the 19 common enzymes involved in the degradation of straw components, *Aspergillus Niger*, *Aspergillus oryzae*, *Rhizopus oryzae* and *Trichoderma reesei* had 3/6/13/5 enzymes missing, respectively. *Aspergillus Niger* produced the most enzymes, and the specific enzyme activity of 11 species from *Aspergillus Niger* was higher than that from *Trichoderma reesei*, which indicates that *Aspergillus Niger* is more suitable as an industrial microorganism to produce enzymatic hydrolysis of straw.

In addition, *Aspergillus Niger* is a common model microorganism for expressing industrial enzymes in which many enzymes have been successfully expressed, such as lignin peroxidase, ferulic esterase and manganese peroxidase [2]. Although the synergistic effect of a variety of microorganisms on the hydrolysis of straw is better than single microorganism, it is difficult to control the growth process. *Aspergillus Niger* can express many exogenous enzymes efficiently after modification. Simultaneous expression of multiple enzymes by *Aspergillus Niger* to hydrolyze straw is becoming a hot research issue.

2.2. Excellent cell factory

Several representative strains of *Aspergillus Niger* have completed the genome sequencing, and established protein secretion pathway, which is conducive to the expression of foreign genes in *Aspergillus Niger*. The protein Ku70 and protein Ku80 of NHEJ (Non-Homologous End Joining) repair pathway in *Aspergillus Niger* were knocked out by optimizing the length of homologous sequence, vector configuration and targeting site in the process of homologous recombination [5]. In addition, *Aspergillus Niger* has been transformed into an excellent cell factory through the development of strong promoter, the construction of protease deficient strains, and systems biology methods [6, 7].

Apart from *Aspergillus Niger* can secrete a lot of kinds of enzyme, it also can express a variety of exogenous enzymes as a cell factory, including xylanase, glycosidase enzymes, Arabian furanose enzyme lipase, methyl pectin enzyme, phosphoric acid, phytase, pectinase, glucose oxidase, lignin peroxidase, glucose and furan glycosidase [8]. The level of fungal enzymes expressed by *Aspergillus Niger* is higher than proteins from other sources, which may be due to the fact that fungal proteins are more suitable for *Aspergillus Niger* in proteolysis and glycosylation [9].

*Aspergillus Niger* has been used as an industrial strain producing citric acid since the 1930s. Now, 99% of citric acid all over the world (1.5 million t/a) is produced by *Aspergillus Niger* [6]. Accumulation of citric acid by *Aspergillus Niger* is accompanied by up-regulation of several enzymes. It can also produce gluconic acid (4.5g/(L·h)), galacturonic acid, and succinic acid [10]. At present, *Aspergillus Niger* metabolic network regulation technology has also been established based on genomic data, which is conducive to the accumulation of citric acid and other metabolites.
2.3. High value-added products

Aspergillus Niger can also produce high value-added compounds by biotransformation of reactions such as hydroxylation, oxidation, reduction, demethylation, vulcanization, dechlorination, ring opening and conjugation [11]. For example, Aspergillus Niger reduces ethyl butyryl acetate to ethyl 3(R) hydroxy caproate with optical purity above 99% [12], and converts heterocyclic compounds, epoxides, aromatic carbohydrates, terpenoids, steroids and flavonoids into drugs and chemical intermediates. With the emergence of a new generation of gene editing technology CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas, the efficiency of Aspergillus Niger gene modification has been rapidly improved [13]. It is reported that transient expression of Cas9 could achieve 100% gene integration, and also demonstrated the great effect of CRISPR/Cas technology on increasing aconitric acid production [14]. A CRISPR/ Cas-based expression system for U6 promoter has been established in Aspergillus Niger [15]. These techniques will accelerate the application of Aspergillus Niger.

3. Enzymes production of Aspergillus Niger in straw degradation

Aspergillus Niger culture with straw as substrate for cellulase production can not only utilize straw effectively, but also produce high value-added products such as enzymes. Because of their high yield, wide application and high safety, Aspergillus Niger-derived enzymes are attracting more and more attention. Aspergillus Niger has become one of the common strains in industrial applications. The commercialized enzymes from Aspergillus Niger include α-amylase, lipase, cellulase, glycosylase, catalase, β-galactosidase, glucose oxidase, hemicellulase, glucosidase, Hesperidase, gluconase, tannase, naringinase, protease, pectinase, etc.

3.1. Cellulase

As the cellulase activity in Aspergillus Niger is higher than that in Trichoderma reesei, it has been widely used in cellulase production. A variety of biomass including straw has been used to culture Aspergillus Niger for cellulase production. For example, groundnut straw and wheat bran as substrates were used to culture Aspergillus Niger to produce cellulase and β-glucosidase whose activities were significantly higher than that of other fungi [16]. Aspergillus Niger strain 3.3148 fermentation increased the crude protein content in corn straw by 1.2 times, and decreased the mass fraction of crude fiber, neutral detergent fiber and acid detergent fiber by 34%, 19% and 23%, respectively [17]. The specific enzyme activities of CMCase and Fpase produced by Aspergillus Niger reached 29.94U/g and 13.56U/g.

3.2. Hemicellulase

As an important part of hemicellulose, xylanase can decompose xylan in the cell wall of straw, reduce the viscosity of materials, promote the release of effective substances, and facilitate the absorption of nutrients. At the same time, xylo oligosaccharides hydrolyzed by xylan can promote the absorption of calcium in acidic drinks and fermented foods. Therefore, xylanase plays an important role in the utilization of straw. Corn cob and wheat bran with high xyleose content are good materials to induce Aspergillus Niger to produce xylanase. The activity of xylanase in Aspergillus Niger B03 cultivated with corncob was increased by 33% [18]. Aspergillus Niger DSM26641 could produce 24.5mu/mL xylanase cultured with palm shell [19]. The results of transcriptional analysis showed that this strain produced three xylanases and two β Xylosidases, four arabinofuranases, one acetylxylanase and two ferulic esterases. The specific enzyme activity of thermostable xylanase produced by Aspergillus Niger SCTCC400264 was 2547.7u/mg, so that the xylanase remained 74% active for 30 minutes at 80 °C [20].

3.3. Lignin-degrading enzyme

There are several possible laccase sequences in the genome of Aspergillus Niger ATCC1015, among which, McoB gene has been proved to express a high amount of laccase [21]. The activity of lignin
peroxidase reached 652.34U/L in *Aspergillus Niger* CGMCC5992 cultured by solid-state fermentation of corn straw [22]. Although *Aspergillus Niger* did not produce manganese peroxidase and diversity peroxidase, it can be used as host to express manganese peroxidase of *Phanerochaete Chrysosporium*, which can degrade phenanthrene in soil.

### 3.4. Pectinase

Pectinase has been used in fruit juice clarification, fruit and vegetable juice extraction, fruit peeling, wood preservative, hemp degumming and cotton fabric refining and other industries [23]. *Aspergillus Niger* can produce three kinds of pectinase: pectin fissure synthase, polygalacturonase and invertase. There are many studies on the production of pectinase by *Aspergillus Niger* using straw. In recent years, researchers have improved the yield of pectinase produced by *Aspergillus Niger* utilizing protoplast mutation, UV mutagenesis and uv/LiCl mutagenesis [2].

### 4. Prospect

The main advantage of *Aspergillus Niger* in straw resourcable utilization is that it has been used in many aspects as a food safety microorganism. *Aspergillus Niger* has abundant self-hydrolyzing enzyme system, and the genetic manipulation technology of *Aspergillus Niger* is mature, which provides technical support for the holoenzyme system of *Aspergillus Niger* to produce hydrolyzing straw components at the same time. *Aspergillus Niger* can accumulate a variety of high value-added substances, and these advantages are helpful for *Aspergillus Niger* to play its role in the straw resourcable utilization process.

Meanwhile, there is still a lot of work to be done in the process of straw resourcable utilization by *Aspergillus Niger*. First, the mechanism of *Aspergillus Niger* regulating the expression of hydrolase is under analyzing. *Aspergillus Niger* expressed different hydrolases in different biomass groups which indicates that *Aspergillus Niger* can sense the composition of substrate and give corresponding feedback. Researches on the transcriptional and expression mechanism are helpful to regulate the expression of hydrolase system of *Aspergillus Niger*. Second, industrial strains expressing holozyme with high efficiency were constructed. In recent years, the emergence of gene editing technology has provided technical support for *Aspergillus Niger* to express a variety of exogenous enzymes. However, there has been no report on *Aspergillus Niger* strains expressing biomass hydrolyzed holozymes. Finally, biological processes are optimized to efficiently utilize biomass. The process of *Aspergillus Niger* utilizing hydrolase to recycle biomass is affected by many conditions in biological process, such as mass transfer and heat transfer, so the control of biological process is also an important research content.

With the deepening of research, *Aspergillus Niger* will play a greater role in biomass resource utilization, which will not only promote the process of biomass resource utilization, but also provide a stage for the application of *Aspergillus Niger*.

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