Decomposition of Maize Straw between Two Phosphate Solubilizing Fungi: *Aspergillus Niger* and *Penicillium Chrysogenum*

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Abstract. *Aspergillus niger* (*A. niger*) and *Penicillium chrysogenum* (*P. chrysogenum*) can significantly promote the degradation of maize straw and phosphorus release. Compared with *P. chrysogenum*, *A. niger* is more efficient in maize straw degradation and phosphorus releasing. After seven days of incubation, the highest degradation ratio and phosphorus content in *A. niger*+maize straw treatment is 25.8% and 2.3 mg/L, respectively. The mechanisms for maize straw decomposition between these two fungi are different. Oxalic acid is the primary organic acid secreted by *A. niger*, which is more function in the decomposition of maize straw compared with propionic acid secreted by *P. chrysogenum*. In addition, *A. niger* has higher acidic xylanase and lignin peroxidase enzymes activities, which is conducive to the degradation of more stable substances in maize straw, i.e., lignin. This study indicated that *A. niger* is the primary candidate for the reuse of crop straw in the way of return to the field.

1 Introduction

Crop straw (CS) is a common residual material produced by the agricultural industry. The production of CS is rapidly increased during the development of agriculture, especially in great agricultural countries. Due to CS contains more than half of the photosynthesis products by crops, it stores large amounts of cellulose and lignin. In addition, CS is also rich in nitrogen (N), phosphorus (P), potassium (K), and other nutrients, which has been recognized as a renewable biological resource with multiple uses [1].

Straw return to fields (SRF) has become an important mode of agricultural resource reuse. In China, more than 5% of CS was treated by the way of SRF every year [2]. This mode not only increases soil fertility (organic matter and NPK) but also improve soil structure and microbial activity [3]. However, the SRF mode is usually limited in the agricultural production process due to the low degradation of CS. The cellulose, lignin and hemicellulose in CS are stable and recalcitrant to degradation, especially for lignin. The degradation ratio of lignin is approximately 30% after one year in nature, which lead to soil pathogens and aggravation of crop diseases [4]. Therefore, the accelerating degradation of straw is an important factor for improving straw utilization and sustainable agricultural development.

Physical, chemical, and biological treatments have been conducted to improve straw digestibility in various researches. Compared with physical and chemical methods, the biological method is environmentally friendly and potentially economically viable alternatives, especially via microbial degradation (e.g., fungi) [5]. On the one hand, the fungal mycelium can destroy the CS cell wall structure, grown inside the parenchyma cells and fibroblasts. On the other hand, the mycelium utilized intracellular substances to secrete the cellulose-degrading enzymes and lignin-degrading enzyme, decomposed cell wall and fibrous tissue, then the cell wall is separated from other substances [6]. In addition, straw incorporation with the fungal is beneficial to the release of available nutrients of N, P and K, hence increasing plant height and yield [7].

The phosphate solubilizing fungi (PSF) *Penicillium* and *Aspergillus* are the common fungi in the soil, which function in the solubilization of insoluble phosphates via secretion of large amounts of organic acids [8]. More importantly, these two fungi can also promote straw degradation [6]. However, the CS decomposition mechanism and P release ratio between these two fungi is scant. Therefore, both *Aspergillus niger* (*A. niger*) and *Penicillium chrysogenum* (*P. chrysogenum*) were selected for the maize straw degradation and its P release ratio in this research. The content of P was determined by an inductively coupled plasma emission spectrometer (ICP-OES). The organic acids secreted by *A. niger* and *P. chrysogenum* were analyzed by high-performance liquid chromatography (HPLC). The enzyme activity of cellulase (CL), acidic xylanase (ACX) and lignin peroxidase (Lip) were determined. The morphology of maize straw and fungi was analyzed by X-ray diffraction (XRD) and scanning electron microscopy (SEM).

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2 Materials and Methods

2.1 Preparation of maize straw and fungal strains

The stalk of fresh maize plants was collected and heated for 30 min at 105 °C and then dried at 65 °C to a constant weight. Subsequently, dried and cleaned maize straw was cut into 2 cm sections for use.

A. niger and *P. chrysogenum* were isolated from maize rhizosphere soil in Suzhou city, Anhui province, China, respectively. After incubated for five days at 28 °C, the spores of *A. niger* and *P. chrysogenum* were collected by sterile water from potato dextrose agar. The suspension was then filtered through three layers of sterile cheesecloth to eliminate mycelial fragments. Spore concentration was calculated and adjusted to 10^7 CFU mL^{-1} by using a hemocytometer [9].

2.2 Fungal incubation with maize straw

The experiment was performed with five treatments, i.e., maize straw (Mst), *A. niger* (ANG), *P. chrysogenum* (PCH), *A. niger*+straw (ANG+Mst) and *P. chrysogenum* +straw (PCH+Mst). Before the incubation, the PVK medium was sterilized at 121 °C for 20 min. Then, 0.2 g straw powder was added to 150 mL conical flasks with 50 mL PVK culture medium, respectively[10]. Subsequently, 1 mL spores of *A. niger* and *P. chrysogenum* were added in the respective treatments. All operations were performed under sterile conditions. The flasks were incubated for seven days at 28 °C, 180 rpm. After seven days of incubation, the PVK medium was filtered through a 0.22 μm polyethersulfone membrane. The straw and microbial hyphae were collected separately. The filtrate was collected for the test of organic acids, soluble P and degrading enzyme activities. The hyphae were frozen dried to determine biomass. The straw was repeatedly rinsed with sterile water, then was dried to degradation rate and to analyse XRD and SEM.

In parallel, a experiment was pursued to detect the P release and degradation of different organic acids on the straw. The experiment was performed with three treatments i.e., maize straw (Mst), oxalic acid+straw (OA+Mst) and propionic acid+straw (PA+Mst). The concentration of OA and PA in each treatment was 2000 mg/L. The culture conditions and subsequent processing were analyzed as described above.

2.3 Degradation rate and degrading enzymes

The straw degradation rate was determined by oven-dried at 65 °C to constant weight, the formula of the degradation rate is as follows:

$$DR(\%) = \frac{Wo-Wt}{Wo} \times 100\% \qquad (1)$$

Wo was the original dry weight of the maize straw and Wt was the dry weight of the straw after incubated for seven days.

The filtrate was collected to analyze CL, ACX and Lip activities via CL, ACX and Lip activity assay kits, respectively (Comin Biotechnology Co., Ltd, Suzhou, China). All the samples were obtained and determined under relevant guidelines and regulations.

2.4 Instrumentation

The pH values were determined using a pH meter (FE28, Mettler Toledo, Shanghai, China). The concentration of the soluble P was analyzed by using ICP-OES (PerkinElmer Avio 200). A calibration curve (0.1, 0.5, 1, 2 and 5 mg/L) was prepared by using the P standard. The R square value of the external standard curve is 0.9999. The contents of organic acids in each treatment were analyzed by HPLC (Agilent 1260). Mineralogical characterization of the precipitates was examined by XRD by using D/Max-2500 X-ray diffraction. The surface morphology of the samples was studied under SEM (S4800 Hitachi) with an acceleration voltage of 10 kV. Detailed methods refer to the previous studies [10].

3 Results & Discussion

3.1 Fungal biomass and P concentration

The fungal dry biomass in Mst, PCH and ANG treatments were 20.3, 11.5 and 10.8 mg on 7th day of incubation, respectively. However, the dry biomass in PCH+Mst and ANG+Mst treatments significantly increased to 299.9 and 418.2 mg after seven days of incubation (Fig. 1). The addition of Mst can significantly promote the growth of fungi. Compared with *P. chrysogenum*, *A. niger* was more efficient in the utilization of Mst.

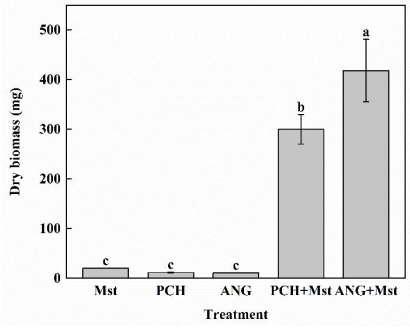

Fig. 1. The concentrations of dry biomass in each treatment. The error bars represent the standard deviations of three replicates.

The soluble P content in Mst, PCH and ANG treatments were 0.63, 0.5 and 0.6 mg/L after seven days of incubation, respectively (Fig. 2). In PCH+Mst and ANG+Mst treatments, the soluble P content was 2.0 and 2.3 mg/L, respectively (Fig. 2). Although both *P. chrysogenum* and *A. niger* promoted the release of P from Mst, *A. niger* showed a higher P solubilizing capacity.

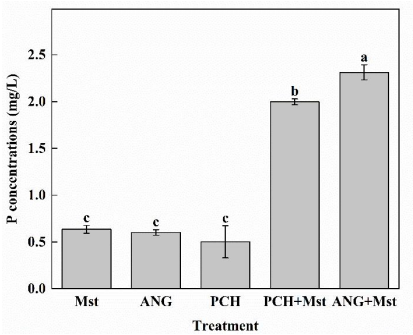

Fig. 2. The concentrations of soluble P in each treatment after seven days of incubation. The error bars represent the standard deviations of three replicates.

3.2 Degradation rate and degrading enzymes

The straw degradation rate in Mst and PCH+Mst treatments was 14.66% and 17.2%, respectively (Fig. 3). In ANG+Mst treatment, the straw degradation rate was 25.86%, significantly higher than other treatments (Fig. 3). Due to P was involved in the formation of the plant cell wall, hence the release of P was also accompanied by the destruction of crop straw [11]. The existence of *A. niger* and *P. chrysogenum* improved the degradation of straw and promoted the release of P.

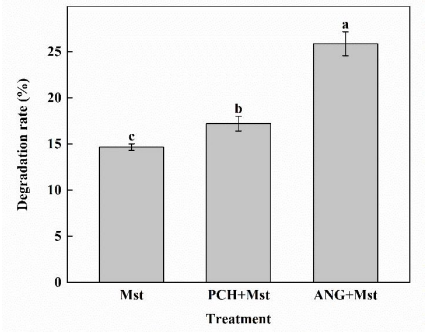

Fig. 3. The degradation rate in each treatment after seven days of incubation. The error bars represent the standard deviations of three replicates.

The activities of degrading enzymes in the treated group (ANG+Mst and PCH+Mst treatments) were significantly higher than others (Mst, ANG and PCH treatments). The CL activity in Mst, PCH and ANG treatments were 348.8, 447.2 and 382.5 nmol/min/mL, respectively (Fig. 4). While the CL activity in PCH+Mst and ANG+Mst treatments was 740.87 and 919.67 nmol/min/mL (Fig. 4). The ACX activity in Mst, ANG and PCH treatments were ranged from 23.3 to 38.6 nmol/min/mL (Fig. 4). In PCH+Mst and ANG+Mst treatments, the ACX activity increased to 45.9 and 169.3 nmol/min/mL treatment, respectively (Fig. 4). The Lip activities in Mst, ANG and PCH treatments were 375.3, 472 and 773 nmol/min/mL, while significantly increased to 1258.3 and 1666.7 nmol/min/mL in PCH+Mst and ANG+Mst treatments (Fig. 4). That is to say, *P. chrysogenum* showed a high CL activity for cellulose degradation, while *A. niger* had high ACX and Lip activities for lignin degradation. Due to lignin was more stable than cellulose, hence *A. niger* was more efficient in maize straw degradation and P dissolution compared with *P. chrysogenum*.

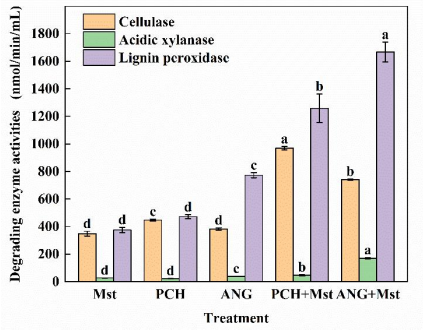

Fig. 4. The degrading enzyme activities in each treatment after seven days of incubation. The error bars represent the standard deviations of three replicates.

3.3 The organic acid secretion by *A. niger* and *P. chrysogenum*

The primary organic acids secreted by *A. niger* and *P. chrysogenum* were oxalic and propionic acid (Fig. 5). In Mst, PCH and ANG treatment, the content of OA and PA had a low value (lower than 200 mg/L) (Fig. 5). In PCH+Mst treatment, the contents of OA and PA secreted by *P. chrysogenum* were 12 and 882 mg/L (Fig. 5). In ANG+Mst treatment, the contents of OA and PA secreted by *A. niger* was 1183 and 200 mg/L (Fig. 5). These two fungi exhibited different organic acid secretion strategies for the same crop straw. The existence of Mst stimulated *P. chrysogenum* to secrete more PA, while *A. niger* secreted more OA. Although both OA and PA had a high capacity of medium acidification and strong metal-complexation activity ability, OA was more suitable for the lignocellulose degradation as the substrate like wheat straw [12]. In addition, oxalic acid was more efficient than propionic acid wood degradation via the formed calcium oxalate crystals in cell walls [13] .

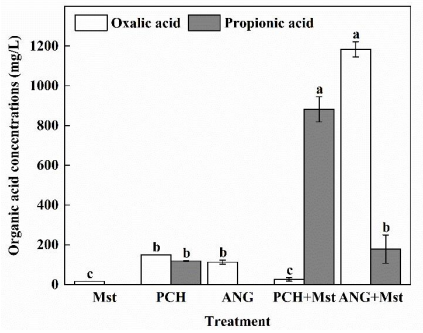

Fig. 5. The secretion of organic acids by *A. niger* and *P. chrysogenum* after seven days of incubation. The error bars represent the standard deviations of three replicates.

3.4 The maize straw degradation and P release by PA and OA

The P release ratio in PA+Mst and OA+Mst treatments was 59.34% and 68.18%, higher than Mst treatment (20 and 28.9 %), respectively (Fig. 6). The degradation ratio

of maize straw in Mst treatment was 30.3% (Fig. 6). In PA+Mst and OA+Mst treatments, the degradation ratio was 44.4% and 51.0% (Fig. 6). Organic acids not only have an essential function in the release of P but also facilitate straw degradation in multiple ways [14, 15]. The initial reaction between organic acid and crop straw involves mild acid-catalyzed hydrolysis of the glycosidic bonds of hemicellulose and the α-ether linkage in lignin. The formed by cleavage of the labile ester groups, catalyze the hemicellulose hydrolysis [16]. PA can loosen the structure of lignocelluloses and cellulose, resulting in an improved overall rate due to the increased accessibility to enzymes [17]. In addition, OA has several roles in chelating unstable Mn^{3+} ions, providing H_2O_2, and lowering pH outside of the fungal hyphae, all of which are important factors for the performance of lignin-degrading peroxidases [12]. Our results confirmed that OA increased the Lip activity of *A. niger*, which was conducive to straw degradation and P release.

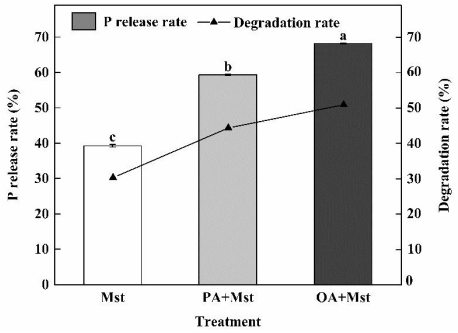

Fig. 6. P release rate and degradation rate of straw in propionic acid (PA) and oxalic acid (OA) treatments.

3.5 XRD and SEM analysis

The spectra of X-ray diffraction showed that the cellulose peaks located at 37.3 and 43.7° were detected in Mst, PA+Mst and OA+Mst treatments (Fig. 7). However, the cellulose peaks were weakly occurred in PCH+Mst treatment and disappeared in ANG+Mst treatment. The new peaks (16.9 and 19.7°) stand for cellobiose and cellotriose (31.03°) were identified in PCH+Mst and ANG+Mst treatments (Fig. 7). This result indicated that cellulose fractions could be transformed to cellotriose and cellotriose by *P. chrysogenum* and *A. niger*.

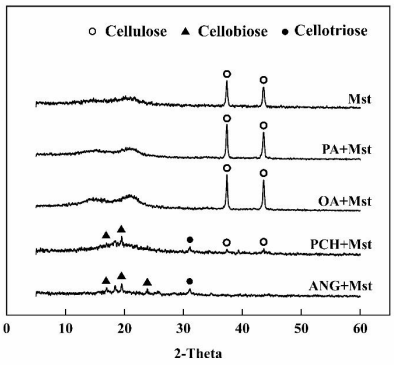

Fig. 7. XRD patterns of the straw in each treatment after seven days of incubation.

SEM imaging of maize straw in different treatments after seven days of incubation was demonstrated in Fig 8. The surface of the original maize straw was dense and smooth (Fig. 8A). After seven days of incubation, the straw also maintained its skeleton despite the presence of holes area in Mst treatment (Fig. 8B). In OA+Mst and PA+Mst treatments, the cellulose skeleton of straw began to collapse, and the broken and split fibres could be observed (Fig. 8C&D). In ANG+Mst and PCH+Mst treatment, the structure of the maize straw matrix was disrupted, both the fungal hyphae and fracture fragments were clearly visible within the straw (Fig. 8 E&F). The results indicated that *A. niger* and *P. chrysogenum* could degrade straw, which was also confirmed by XRD results.

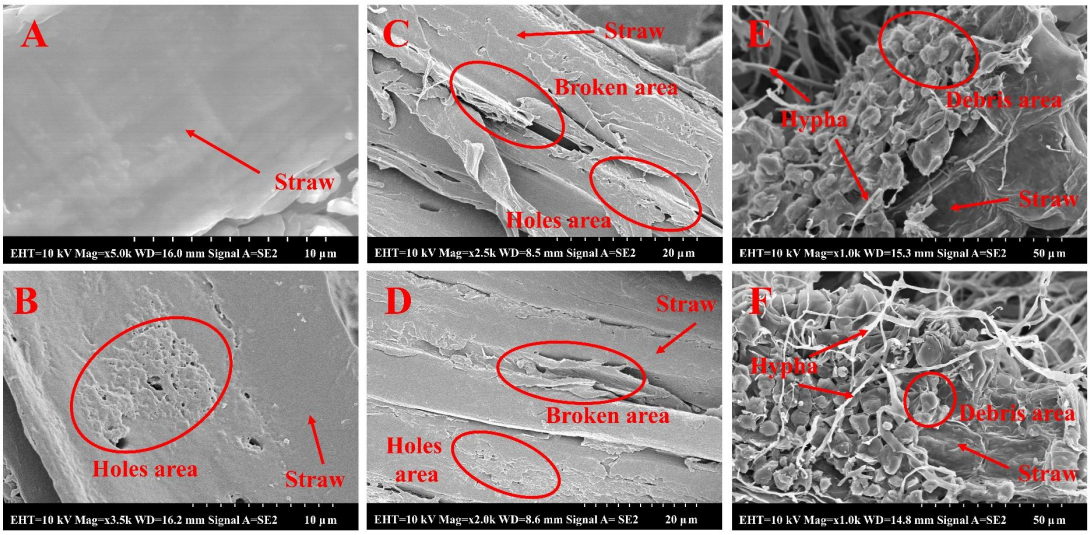

Fig. 8. SEM imaging of the precipitates from maize straw in different treatments after seven days of incubation. A: maize straw before incubation; B: maize straw after 7 days of incubation; C: OA+Mst; D: PA+Mst; E: ANG+Mst; F: PCH+Mst.

4 Conclusion

This study indicated that both *A. niger* and *P. chrysogenum* can promote maize straw degradation and P release. However, *A. niger* is more effective compared with *P. chrysogenum* due to the different enzymes and organic acids production. Although *P. chrysogenum* has a high CL activity, the ACX and Lip activity in *A. niger* is higher, which is more efficient in the degradation of stable lignin in maize straw. In addition, the dominant oxalic acid secreted by *A. niger* is also more efficient than propionic acid secreted by *P. chrysogenum* in maize straw degradation and P release. These results indicate that the mechanisms of maize straw decomposition and P release are different between *A. niger* and *P. chrysogenum*. *A. niger* should be considered as the primary candidate for the crop straw return to the field.

This work was supported by the program at Department of Natural Resources of Anhui Province (NO. 2021-K-11), the National Natural Science Foundation of China (NO. 42007030 and NO. 41877099) and the program at Anhui Agricultural University (NO. yj2019-20).

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