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Study on Degradation of Rape Straw by Cellulose Degrading White Rot Fungus

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Abstract. In order to transform rape straw into an effective animal feed, white rot fungi were isolated from rape straw buried under soil for about 30 days, and the effects of white rot fungi and culture formula (1, 2 and 3) on cellulose degradation rate and crude protein level in rape straw was studied. The results showed that 35 white rot fungi were isolated from rapeseed stalks buried under soil for about 30 days, among which white rot fungus strains LX03 and LX08 grew fast and had high D/d values. Two white rot fungi (LX03 and LX08) were inoculated separately into three solid culture formulations, and the results showed that both the degradation rate of cellulose and the crude protein content in rape straw fermented material by LX03 and LX08 had an increasing trend with the increase of fermentation time. The degradation effect of strain LX03 on rape straw cellulose was better than that of strain LX08. Compared with Formulation 2 and Formulation 3, Formulation 1 was more conducive to the growth of strain LX03 and the degradation of cellulose ($P<0.01$).

INTRODUCTION

China is the world's largest rapeseed producer. Rape straw is the main by-product of rapeseed production with 1.5 ratio of grass to valley (the ratio of rape straw yield to rapeseed yield) [1]. According to the statistics of the Food and Agriculture Organization of the United Nations (FAO), the total output of rapeseed in China in 2013 was 14.4 million tons [2], and the converted rape straw was about 21.6 million tons. Rape straw is rich in organic matter such as cellulose, hemicellulose, lignin and protein [3]. Rape straw is an important biological resource, but the lignin in the straw is poor in water solubility, and the structure is dense and stable, and hard to be degraded [4]. In order not to affect the growth of the crops, the rape straw is mainly burned, the utilization rate is low and the environment is polluted [5].

Oilseed rape or Brassica campestris is the most important oil crops in Leshan, Sichuan province, China.The flowers of rape can be seen, rapeseed can eat, and rape straw is an important renewable resources in rural areas. Based on its plant characteristics, chemical composition, nutritional characteristics, material characteristics and other indicators, rape straw has a very high value and development potential in resource-based use [6-7]. Except for a small number to return to the field as a fertilizer and to cultivate edible fungi, most of rape straws in Leshan area did not been effectively utilized, or abandoned or burnt, wasting resources and causing serious air pollution [8]. Therefore, reasonable and efficient use of rape straw is not only great significance for farmers to increase income and resource utilization, is also important for environmental protection.

The use of microorganisms degrading lignin (generally white rot fungi) to decompose lignocellulosic biomass for further utilization is a better method for utilizing agricultural product production or processing by-products [9]. Inoculation of crop straw with white rot fungi and production of fungus feed has undoubtedly a broader development prospect [10-13], which avoids the new pollution caused by other straw treatment methods and greatly enriches the animal's feed source. Due to the higher waxiness, silicate, cell wall crystallinity and the structure of solid ester bond between lignin and cellulose, the digestibility of animals is extremely low, plus odor, coarse and palatable. According

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to reports, the lignin content of rape straw is higher than that of common crops such as wheat and straw [14], which undoubtedly increases the difficulty of converting rape straw into feed.

In view of the actual situation in the region, two white rot fungi LX03, LX08 and three different formulations were screened and used to compare the degradation of rape straw. The purpose of this study was to investigate the biodegradation effect of different fungi and cultivated materials on cellulose in rape straw.

MATERIALS AND METHODS

Separation and Purification of Cellulose-Decomposed White Rot Fungi

The medium for the isolation of strains was Congo red-carboxymethyl cellulose sodium [15]. Collected the rape straw samples buried in the soil for about 30 days, and diluted to 10^{-5}, and taken 0.1 mL the dilution of each sample 10^{-3}, 10^{-4} , 10^{-5} , respectively, were coating inoculated on Congo red-carboxymethyl cellulose sodium medium, and three groups were repeated. After 5 days of culture at 30°C, the diameter of colony growth (d) and the diameter of the transparent circle around the colony (D) were measured. D / d was calculated.The two strains with the largest D / d ratio were further isolated and purified, and stored at low temperature on the slope, which was used for the degradation of rapeseed straw cellulose.

Rape Straw

The rape straw used in this experiment was the next year rape straw in Jiajiang County, Leshan City, Sicuan Province, China. The cellulose content of the rape straw was determined to be 40.25%. Used fresh, dry, clean, mold-free rape straw. Cut the rape stalk into 1 cm pieces, soaked it in 0.5% lime water for 10 h, and rinsed with water to pH value 7, wring it out until it was not dripping, sprinkled it, mixed it with lime, phosphate fertilizer, white sugar and wheat bran. The water content of the culture material was controlled at about 65%.

Test Design

The test was a two-factor completely random combination design. The test was divided into 6 treatment groups with 3 replicates per treatment. Each treatment combination (Table 1) and medium formulation (Table 2) was as follows.

TABLE 1. Six test combinations for test culture

Treatment	Strain	Selection formula
A	LX03	Formula 1
B	LX03	Formula 2
C	LX03	Formula 3
D	LX08	Formula 1
E	LX08	Formula 2
F	LX08	Formula 3

TABLE 2. Formulas of three medium (percentage)

Treatment	Rape straw	Lime	Superphosphate	White sugar	Wheat bran
Formula 1	76.5	1.5	1	1	10
Formula 2	86.5	1.5	1	1	0
Formula 2	87.5	1.5	1	0	10

Fermentation Method

The small straw grasser was used to break up the rape straw into pieces of about 0.5cm in length, baged (the bag length was about 15 cm, the loading was about 0.5 kg), and sterilizated (Sterilized at 127°C 2 h), cooled to about 25 °C , inoculated in sterile room (inoculum volume 2%), fermented at 28°C for 40 days.

Test conditions was Ambient temperature 28 °C, humidity 65% -70%, pH 6-7, protected from light, ventilation.

Determination Method and Indicators

Pretreatment of samples was drying at 80 °C for 3 - 4 h, cooling and pulverizing through a 40 mesh sieve to be tested. The protein content was determined by Kjeldahl's method, and cellulose content was measured by Van Soest's method [17].

Statistical Analysis

The test statistics were statistically analyzed using DPS7.05 completely randomized single factor test and differential significance test was performed.

RESULTS

Screening of Cellulose Decomposing White Rot Fungus

A total of 35 strains with cellulolytic ability were isolated from the collected samples, most of which belonged to fungi, and a few belonged to bacteria and actinomycetes. The purified fungi were planted on Congo red cellulose plates and compared after many experiments. Eight strains with larger diameters of transparent circles were obtained. The relevant traits are shown in Table 3. The two strains LX03 and LX08 with the largest D/d were selected for the degradation experiments of rapeseed straw cellulose.

TABLE 3. Related traits of cellulolytic white rot fungi

Strain	Colony diameter (d, mm)	Transparent circle diameter (D, mm)	D/d	Colony color	Speed of growth
LX01	7	20.2	2.9	milky	general
LX02	3.5	14.4	4.1	light yellow	slow
LX03	8.2	38.4	4.7	green	fast
LX04	7.3	17	2.3	white	general
LX05	4.2	13.1	3.1	milky	slow
LX06	6	17.5	2.9	Grayish white	general
LX07	3.5	12	3.4	white	slow
LX08	8	37.5	4.7	Light brown	fast

Effect of Different Treatments on Mycelial Growth

The effects of different treatments on the mycelial growth of white rot fungi were significantly different (Table 4). It can be seen from Table 4 that under the same formula, the growth rate and hyphae texture of strain LX03 were better than LX08. Mcelial growth of white rot fungi in Formulation 1 (Treatment A and Treatment D) was better than that in Formulation 2 (Treatment B and Treatment E) and Formula 3 (Treatment C and Treatment F), and the difference between Formulation 2 and Formulation 3 was not significant.

TABLE 4. Mycelial growth of each treatment group

Treatment	Strain	Formula	Repeat number	Full bag time (d)	Mycelium growth	Hyphae texture
A	LX03	Formula 1	3	14	++++	Thick, white, and vigorous
B	LX03	Formula 2	3	16	+++	Thick, white, and vigorous
C	LX03	Formula 3	3	16.5	++	Thick, white
D	LX08	Formula 1	3	15.5	+++	Thick, white, and vigorous
E	LX08	Formula 2	3	17.5	+	Sparse, grayish white
F	LX08	Formula 3	3	18	+	Sparse, grayish white

Degradation effect of white rot fungi on cellulose in rape straw

On the 20th day of continuous solid fermentation of rapeseed straw, the degradation rate of cellulose was significantly different among different strains and formulations (Table 5). Degradation ability of cellulose in rape straw of strain LX03 was significantly better than that of strain LX08 (P<0.01) (Table 5). On the 40th day of continuous solid fermentation, the degradation rate of cellulose in each treatment group was improved to some extent. The ability of strain LX03 to degrade cellulose was better than strain LX08 (P<0.01) (Table 5).

TABLE 5. Degradation rate of rape straw cellulose by white rot fungus for 20 days and 40 days (%)

Treatment	Strain	Formula	Repeat Number	20 D	40 D
A	LX03	Formula 1	3	7.92aA	42.09aA
B	LX03	Formula 2	3	5.91cC	23.30bB
C	LX03	Formula 3	3	6.27bB	21.63cB
D	LX08	Formula 1	3	3.61dD	16.83dC
E	LX08	Formula 2	3	3.19eE	14.68eD
F	LX08	Formula 3	3	2.99fF	15.44eCD

Note: Different lowercase letters in the same column of the same column indicate significant difference (P<0.05).Different uppercase letters in the same column indicate significant difference (P<0.01).

3.4 Effect of white rot fungi on crude protein level in rape straw

The crude protein content in rape straw fermented material effectively was increased by white rot fungi (Table 6). On the 20th day of fermentation, the crude protein content of each treatment was increased to some extent, and the treatment A of strain LX03 was significantly higher than that of other treatment groups (P<0.05). On the 40th day of fermentation, the crude protein in each treatment group increased to different extents. The crude protein of each treatment (treated A, B, C) of LX03 was significantly higher than that of LX08 (treatment D, E, F) (p <0.01), indicating that white rot fungus LX03 was more effective than LX08 to promote the production of bacterial proteins, thereby improving the crude protein level of rape straw (Table 6).

TABLE 6. Changes of crude protein content in rape straw at 20th day and 40th day (%)

Treatment	Strain	Formula	Repeat number	20 d	40 d
A	LX03	Formula 1	3	16.37aA	87.80aA
B	LX03	Formula 2	3	13.39bB	27.28cC
C	LX03	Formula 3	3	13.00bcBC	18.75eE
D	LX08	Formula 1	3	12.20cC	74.70bB
E	LX08	Formula 2	3	12.70bcBC	18.35eE
F	LX08	Formula 3	3	12.70bcBC	23.02dD

Note: Different lowercase letters in the same column of the same column indicate significant difference (P<0.05).Different uppercase letters in the same column indicate significant difference (P<0.01).

DISCUSSION AND CONCLUSION

The cellulose degradation rate was relatively low on 20th days in solid fermentation time, and the degradation rate was greatly improved on 40th days. This was because the strain gradually destroyed the plant cell wall with the prolongation of fermentation time. The composition of lignin, so that the late stage cellulose degradation rate would be greatly improved. Different edible fungi had different abilities and processes for degrading cellulose [11]. The growth stage of *Lentinus edodes* can make good use of lignin, while *Volvariella volvacea* was another type fungi that cannot use lignin, but can make good use of cellulose. In this experiment, the strain with strong enzyme production ability and the medium suitable for inducing ligninase production were used for solid fermentation to achieve good

degradation of cellulose. The strain LX03 grew fast and grew well, and degraded cellulose in rape straw. It has more advantages and potential than strain LX08, and it was worthy of selection and production promotion.

The results of this experiment indicated that the crude protein content in rape straw fermented material could significantly increase through the cultivation of white rot fungi, which may be due to the fact that the non-protein nitrogen in the culture material was gradually converted into a large amount of mushroom protein under the action of white rot fungi. Among them, the formula 1 of the strain LX03 particularly improved the crude protein.

In this experiment, the degradation of rape straw cellulose by white rot fungi was not as prominent as some experiments reported [18-20]. It may be because the physical and chemical properties of rape straw itself are different from other straws, and the poor nutrition and nitrogen deficiency cause C /N imbalance and other nutrient deficiency will affect the vegetative growth rate of white rot fungi and the degradation of water-insoluble carbohydrates. Further testing is needed to explore suitable cultivation parameters.

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