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Straw bio-degradation by acidogenic bacteria and composite fungi

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Abstract: A composite microbial system, including a strain of *Candida tropicalis*(*W*3), a strain of *Lactobacillus plantarm*(*WY*3) and three strains of *basidiomycete pL*104, *pL*113 and *C*33, was chosen to degrade corn straw. The final pH was acid owing to the inoculation of acidogenic bacteria, and under this condition the composite fungi system could produce complex enzyme to destroy the compact structure of corn straw. The experimental results showed that the biomass of composite fungi could reach up to maximum when the pH value was 4.5. Through the bio-degradation by combining acidogenic bacteria with the composite fungi system, the cellulose, hemi-cellulose and lignin degradation rates of corn straw powder were 26.36%, 43.30% and 26.96%, respectively. And the gross crude protein content increased 60.41%. This study provided the evidence for the feasibility of developing a composite microbial system with high capability of degrading straw lignocelluloses in order to make reasonable use of straw resource and protect rural eco-environment.

Keywords: lignocellulose; bio-degradation; acidogenic bacteria; composite fungi system

Introduction

The annual amount of straw was 2—3 billion tons in the world. In China, the annual amount of all kinds of straws, accounting for 20%—30% that of the world(Guo, 2003), was up to 0.62 billion tons. Straw is not only a by-product in agro-farming, but also an important bio-resource. It is estimated that one half of harvest is the seed and the other is the straw during corn production(Ding, 1993). The straw consumes the coordinative nutrients of the soil and needs human coequal cost. Therefore, these huge materials should not be ignored. However, it is rather difficult to make full use of these resources; the main reason is that the straw production changes at different seasons with the characteristics of large amount, low value and huge volume. Most animals can not digest its lignocellulose. The natural degradation process is also very slow. A large amount of straw run into the natural environment in the pattern of throwing aside as solid waste or setting on fire except a fraction of straw used as fuel, fodder, manure, raw materials for paper making, building and textile, are of low utilization ratio, low transformation ratio, low economic efficiency. As a result, it brings about regeneration resource waste, obstructs traffic and causes serious rural eco-environment pollution. What is more, unreasonable disposition of crop straw has become an important source of rural non-point pollution. Therefore, it is urgent to find an ideal utilization way for large amounts of straw.

The cellulose content in straw is up to over 30%, while the crude protein content is only 2%—8%, lacking in mineral and vitamin. As forage, the straw is difficult to digest in addition to its large volume, hard character and bad appetite. However, if the nutritive value of straw, with 70% carbohydrates (Chen, 1997), is improved by adopting a series of advanced techniques, the straw could be changed into energy feed of domestic animals.

Until now, a series of straw treatment methods have been studied. The appetite for straw can be improved, the consumption of energy and the waste of forage in the feeding process can be decreased, but the straw digestive efficiency and the amount of available nutrient have not been increased by physical treatment methods such as cutting up, grinding, maceration, cooking and inflating. While, through chemical treatment methods such as aminating, basification, acidification etc., the suitable feeding of straw forage can be improved, but the question of transferring the cellulose into digestive nutrient has still not been solved. The bio-treatment methods, including microbial fermentation, enzymatic decomposition etc, are becoming a main field because they not only improve the nutrient value of straw forage in a large scale but also possess the characteristics of economizing natural resources and lessening environmental pollution.

In 1940's—1950's, the cellulose and its related decompounded mechanism of straw's three varieties of non-nutrient materials became the study focus all over the world, and in 1980's—1990's, the study on straw *Lignocellulose* degradation stepped into a new era owing to the founding of lignose enzyme(Shi, 2002). At present, the decomposing mechanism and degradation enzymes are the key difficult points in technique study. The main reason is that straw's three varieties of non-nutrient materials have special compact structure and no single decomposing enzyme could complete the whole degradation process(Nadia, 1997).

It is well known that microbes play an important role in decomposing organic matter and are regarded as human environment dustmen. But in the past decades, bacteria, *actinomycete* and little fungi are applied most, huge fungi are utilized less in the microbial production (Li, 2002). The recent studies showed that cellulose produced by bacteria belongs to endo-enzyme and is absorbed in the cell wall, as a result, bacteria are seldom used as seeding strains of cellulose enzyme production. *Mildem*, *wooden mildem genus*, is recognized as the production strain of cellulose, however, they could not be applied in practice as forage due

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to air pollution from fungi spore after being dried. The majority of huge fungi, especially *basidiomycete*, belong to saprophytic fungi, they get nutrient and energy by decomposing defoliate, dead wood, straw, dejection and other organic matter. All saprophytic fungi play a vital role in the natural matter cycle owing to the degradation ability of cellulose, semi-cellulose and lignin. From the point of view of microbial ecology principle, the degradation of special organic matter is realized by multi-microbes (Rodriguez, 1996; Broudiscou, 1999).

The microbial fermentation treatment of straw meets the challenge: firstly, the straw cellulose, lignin and waxiness, integrating together, result in decreasing enzymatic active ability; secondly, it is difficult to screen high efficiency strains of cellulose enzyme production; thirdly, it is vital to solve the feedback restrain of fermentation production to synthesize the enzyme. Therefore, the recent studies of straw bio-degradation stated that the function of co-culture of different microbes was emphasized gradually. In this study, different high efficiency microbes were screened and combined to decompose the straw without destroying the coordinate relationship of natural microbes (Cui, 2002; Dorado, 1999). This paper reported the straw bio-degradation result and ecological characteristic by using acidogenic bacteria and composite Fungi

1 Materials and methods

1.1 Microbial strains and their source

Test strains: a strain of *Candida tropicalis* (*W*3), a strain of *Lactobacillus plantarm* (*WY*3) and three strains of *basidiomycete pL*104, *pL*113 and *C*33, with considerable capability of lignin-cellulose degradation. All these strains were screened by Microbial Lab of Tianjin Agro-Biological Technique Research Center.

1.2 Culture medium

Bevel solid culture medium tube: McClary's medium, PDA synthetic medium, lactobacillus synthetic medium; liquid culture medium: McClary's liquid medium, PDA medium, lactobacillus liquid medium; solid fermentation culture medium for fungi: straw: base culture medium = 1:3, a little $C_6H_{12}O_6$ and little $MnSO_4$, $(NH)_2SO_4$ in addition; solid fermentation culture medium for co-culture of microbes: corn straw powder which passed 18 fine screen 80%, corn flour 15%, wheat bran 5%, and moisture 65%.

1.3 Experimental methods

Test on single strain of fungi for degrading straw cellulose, hemi-cellulose and lignose: 10 ml suspended liquid of three *basidiomycete pL*104, *pL*113 and *C*33 were inoculated into solid fermentation medium for fungi, respectively, cultivated at 30℃, this test was designed with three repeats, two controls, respectively; the amounts of straw cellulose, hemi-cellulose and lignose were monitored respectively after 10 d cultivation, the detailed results (average value) are shown in Table 1.

Table 1 The degradation efficiency of the cellulose, hemi-cellulose and lignin of corn straw powder by single strain of *basidiomycete*

Strains	Time, d	Hemi-cellulose, %	Cellulose, %	Lignose, %
*pL*104	10	12.2	4.1	27.1
*pL*113	10	14.2	13.1	23.13
*C*33	10	11.5	21.3	14.7

Test on the relationship between pH and the biomass of three *basidiomycete pL*104, *pL*113 and *C*33. The pH values of PDA liquid medium were adjusted into 6 different grads, the other ingredients were not changed at all. 10 ml suspended liquid of three *basidiomycete pL*104, *pL*113 and *C*33 were inoculated into PDA liquid culture medium, the biomass of three *basidiomycete pL*104, *pL*113 and *C*33 were monitored through centrifugation after 10 d cultivation, all these samples were designed with three repeats. The result of the relationship between pH and the biomass of three *basidiomycete pL*104), *pL*113 and *C*33 (average value) is shown in Fig.1.

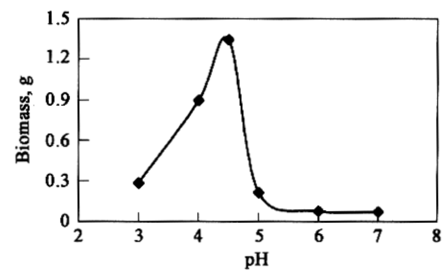

Fig.1 The relationship between pH value and the biomass of a composite fungi system

Test on corn straw powder degradation through co-culture of a strain of *Candida tropicalis* (*W*3), a strain of *Lactobacillus plantarm* (*WY*3) and three strains of *basidiomycete pL*104, *pL*113 and *C*33. This test was designed as follows: control, treatment 1 (inoculation of there strains of *basidiomycete pL*104, *pL*113 and *C*33, its propotion: 1:1:1), treatment 2 (inoculation of a strain of *Candida tropicalis* (*W*3), a strain of *Lactobacillus plantarm* (*WY*3) and a strain of *basidiomycete pL*104), treatment 3 (a strain of *Candida tropicalis* (*W*3), a strain of *Lactobacillus plantarm* (*WY*3) and three strains of *basidiomycete pL*104, *pL*113 and *C*33). Each treatment was inoculated into solid fermentation culture medium for co-culture of microbes and had two repeats respectively. 7 d after cultivation at 28℃, the degradation efficiency of cellulose, hemi-cellulose, lignose and crude protein of corn straw powder were analyzed, the monitored result is shown in Table 2, the electron micro-photos of control sample and treatment 3 were observed and analyzed, as shown in Fig.2.

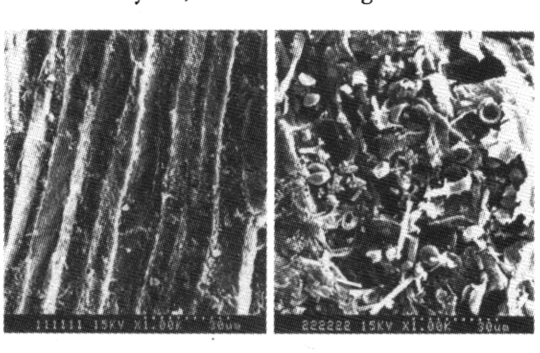

Fig.2 Electron microscope photos of corn straw powder (1500 ×, the left: non-treatment; the right: treatment)

1.4 Mensuration

Van Soest method was adopted to determine the samples' contents of cellulose, hemi-cellulose, lignin (Li, 2001); the fungi biomass was obtained through the procedure of centrifugation, collation, drying, weighing (Cheng, 1981); WTW inoLab pH meter; the crude protein was determined by semi-micro Kjeldahl method (Li, 2001); the method of projection was adopted to observe and shoot the electron micro-photos by electron microscope (Hitachi X-650).

2 Results and discussion

2.1 The degradation efficiency of the cellulose, hemi-cellulose and lignin of corn straw powder by single strain of *basidiomycete* (Table 1)

The final result showed that three strains of *basidiomycete* all could degrade the cellulose, hemi-cellulose and lignose of straw. As far as *basidiomycete* *p*L104, *p*L113 were concerned, the lignose degradation efficiency was up to 27.1% and 23.13% respectively. The cellulose degradation rate for *basidiomycete* *C*33 was 21.3%. All three strains of *basidiomycete* *p*L104, *p*L113, *C*33 belonged to white rot fungi. It was reported that they had no choice for substrate in the process of straw treatment and had coordinate effect (Cui, 2002).

The results demonstrated that pH was an important influence factor for fungi biomass. Fungi was cultivated under the condition of 30℃, 180 r/min, when the pH value was 4.5. The biomass of a composite fungi system increased up to the maximum after 5 h. The authors considered that the three strains *of basidiomycete pL*104, *pL*113 and *C*33 were apt to reproduce in the tiny acidy environment with pH value of 4—5. If co-culture of different microbes was adopted to treat straw, a strain of *Candida tropicalis* (*W*3), a strain of *Lactobacillus plantarm* (*WY*3) could produce a large amount of organic acid, then the pH of fermented substrate would decrease from 6—7 to 4.0 rapidly in the early fermented stage. This case would benefit the fermentation of a composite fungi system.

2.2 Straw bio-degradation by combining acidogenic bacteria and a composite fungi system

As shown in Table 2, the cellulose, hemi-cellulsoe and lignose degradation efficiency of straw treated by combining acidogenic bacteria and a composite fungi system were up to 26.36%, 43.30% and 26.96%, respectively. The crude protein increased from 3.5% to 5.6%, 60.41% of increase. The treatment efficiency was better than that of treatment 1 and treatment 2. The possible function mechanism was: first, the linked bond between hemi-cellulose and lignin of corn straw cell wall was broken up so that a part of lignin was dissolved and cellulose tended to become digestive; second, the straw cell wall was made to expand so that hole degree among celluloses, surface area and water absorption ability of cell wall were increased, and it was useful for the contact between complex enzyme and cellulose or lignin; finally, the aldehyde substance of straw cell wall was reduced (Xi, 2003).

Table 2 The degradation efficiency of corn straw powder under different co-culture of acidogenic bacteria and a composite fungi system (unit: %)

	Control	Treatment 1	Degradation effiecinecy	Treatment 2	Degradation effiecinecy	Treatment 3	Degradation effiecinecy
Cellulose	34.82	31.56	9.36	28.53	18.06	25.64	26.36
Hemi-cellulose	21.57	18.96	12.10	19.35	10.29	12.23	43.30
Lignose	11.72	10.91	6.91	11.42	2.56	8.56	26.96

By means of scanning electron micro-graph (SEM), the original structure of corn straw tissue was compact and in order without any treatment. Compared with the control sample, the tissue structure of corn straw treated by co-culture of microbes did not exist at all and the structure of cell wall in straw was destroyed and became a large amount of inanition (Fig.2). It was estimated that the cellulose, hemi-cellulose linked in lignin of straw might dissociate after bio-degradation by combining acidogenic bacteria and a composite fungi system. Therefore, the cell wall and its content of straw would have more chance to meet microbial enzyme, it would lead to enhance the degradation efficiency of straw.

In microbial ecological system, the inter-relationship of microbes was rather complicated, although different species had different psychological character, they could promote each other in given environment. This case resulted in material cycle and environmental purification. In recent years, the study and application on co-culture of microbes have achieved a lot based on micro-ecology theory. The main aim of this study is to set up a composite microbial model of degrading lingo-cellulose.

In this study, acidogenic bacteria were introduced to decrease the pH of fermented medium so as to create available environmental condition for a composite fungi system and degrade the lignocellulose of straw in a short time. In practice, the straw was decomposed to some extent at low cost and its nutritive value was improved finally.

3 Conclusions

The results of the study showed that it was evident that the cellulose decomposing microbes could promote cooperation, co-culture of many strains leaded to microbial community which could degrade the lingo-cellulose of straw. On the other hand, the decomposing activity of these microbial communities was always restrained because the pH value of inoculating system was unstable (high or low). In the past fermented procedure, acid or alkali material was doomed to add for adjusting the pH value. In this study, in the premise of maintaining the coordinating relationship of different microbes, different microbial strains were reset. As a result, high efficiency co-culture microbes with decomposing straw were obtained.

The method of straw bio-degradation by combining acidogenic bacteria and a composite fungi system was not only to improve the crude protein but also to avoid the metabolism production restraining the activity of cellulose enzyme. Therefore, the co-culture technique of composite microbes leaded to a new way of exploiting protein resource from straw. As far as the choice of fermented microbes is concerned, a composite microbial system is inclined to be selected. It was reported, the protein content for composite fermentation is higher than that of single fermentation. The transformation rate of cellulose is up to 50% according to the calculation of cell production, being close to the theoretic value. This indicates that most of them are utilized. Moreover, the surplus carbohydrate is used by yeast in the proceeding of composite fermentation(Shi, 2002).

In this study, the strains of *basidiomycete pL*104, *pL*113 and *C*33 with considerable capability of cellulose degradation were selected to degrade corn straw, and were safe in the feeding process (Guo, 2003). This would be available for domestic animals in China.

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