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ISOLATION AND IDENTIFICATION OF CELLULOSE-DECOMPOSER FUNGUS *PENICILLIUM OXALICUM* AND DEHYDRATION EFFECTS OF ANALOG COMPOSTS

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ABSTRACT

The aim of this study was to screen cellulose-decomposer microorganisms and to study the effects of dehydration in simulated composting. Cellulose-decomposer microorganisms were first isolated from fresh cow dung and cow dung natural compost in different stages by the cellulose-Congo Red medium. The obtained strains were further screened for a high ability to decompose cellulose by detecting the cellulose-enzyme activity and by observing the disintegration of filter paper and straw. A strong cellulose-decomposer microorganism was finally obtained and was identified as *Penicillium oxalicum* F12. The following aspects were experimentally determined to be the optimal conditions: a wheat bran and microcrystalline cellulose mix (4:1) as the carbon source, ammonium sulfate as the nitrogen source, pH of 5~6, 5% inoculum size, incubation at 30~35ºC for 120h. Under these conditions, the CMCase of strain F12 was 47.50 IU·mL-1, and the FPA was 11.10 IU·mL-1. In a dehydration test of simulated composting, the water content of the inoculated processing residue material was decreased by 3.9% as compared with that of the control residue materials. The dehydration volume was 47.6g, which accounted for 19.8% of the initial material moisture content. *Penicillium oxalicum* F12 was a strong cellulose-decomposer microorganism with broad development potential.

Keywords: cellulose-decomposer microorganism; *Penicillium oxalicum;* simulated composting

INTRODUCTION

With the rapid development of animal husbandry industry in China, the sheer number of livestock and poultry increases with each passing year. Furthermore, research has shown that in China in 2003 the excrements produced by livestock and poultry amounted to 3.190 billion tons, and this amount tends to increase every year (Wang *et al*., 2006a). Therefore, solving the pollution problem of livestock and poultry breeding is a very arduous task of agricultural diffused pollution and ecological environment protection.

As such, composts are one of key technological means in the non-pollution disposal of the excrement produced by livestock and poultry. However, in the non-pollution excrement disposal, the high water ratio, especially that of pig and cow manure, is the bottleneck of quick heating fermentation. In addition， while composts have many various techniques, effectively and rapidly lowering the water contents in wet excrement while simultaneously preserving the relatively high nutrient contents remains a technological difficulty in compost production (Huang *et al.*, 2003). Traditionally, the primary method of reducing the initial water ratio of composts is to add auxiliary material, but this method has its limitations, with an important one being that this method decreases the quality of organic fertilizer. For instance, the fresh excrement of dairy cattle has a high water ratio and a low nutrient content, so fermenting by adding extra auxiliary material in order to adjust the water ratio will ensure that the fermented composts will not achieve the relevant national standards of organic fertilizer. Moreover, common auxiliary materials, such as sawdust and rice chaff, are becoming increasingly more expensive. Additionally, because the price of coal has been increasing ceaselessly in recent years, both sawdust and rice chaff are being used as alternative energy sources to coal. Therefore, using them as the auxiliary material of compost production is costly, and those materials are becoming more difficult to collect. Thus, it is imperative to improve the technology of compost production. Research shows that the water content of both pig and cow manure is caused by the compact net structure that is primarily formed by fiber and colloid and which absorbs a great amount of water (Fei *et al.*, 2006, Chang *et al*., 2007). However, inoculating them with cellulose-decomposer fungus may solve the existing difficulty in the disposal of compost wastes (Adsul *et al*., 2007, Sun *et al*., 2007). This experiment sifts strong cellulose-decomposer fungus from cow dung and composts of different stacking stages and aims at developing suitable microorganism inoculums, improving the initial water ratio, reducing the usage of auxiliary materials, and enhancing the quality of organic fertilizer.

MATERIALS AND METHODS

Screening Methods of Cellulose Decomposition Fungus (Wang . *et al*., 2006b， Wang. *et al*., 2010b).

Isolating Samples: Forty-six samples of fresh cow dung were collected from different compost fields and at different natural compost stages in several organic fertilizer enterprises in Zhejiang Jinhua, Shaoxing, Shangyu, Ningbo, and Hangzhou of China.

Enrichment: The enrichment medium ($g \cdot L^{-1}$) consisted of the following: NH_4Cl 1.1, K_2HPO_4 1.0, NaCl 0.5, KCl 0.2, $MgSO_4 \cdot 7H_2O$ 0.2, $FeSO_4$ 0.001, $CaCl_2$ 0.01, CMC-Na 25, and pH 7.0. First, 5g of the sample was accurately weighed, and then, the sample was diluted and shaken in a triangle flask that contains 100 ml of sterile water with glass beads. Next, 5 ml of the liquid supernatant was removed and added to the enrichment medium and then cultured at 28ºC and 150 rpm for 5 days. The enriched culture was inoculated into fresh medium. This process was repeated 3 times continuously so that the cellulose degradation fungus in the medium reached a high concentration.

Primary Screening: The following constitutes the fungus primary screen medium (PSM, Cellulose-Congo red medium) ($g \cdot L^{-1}$) (Zhang *et al*., 2004): $(NH_4)_2SO_4$ 2.0, $MgSO_4 \cdot 7H_2O$ 0.5, K_2HPO_4 1.0, NaCl 0.5, microcrystalline cellulose 2.0, Congo red 0.4, and agar 16,pH is not adjusted. First, 10 mL of the cultivation solution was removed from the shaken flask that contained the enrichment medium; the viscosity of which had been reduced greatly. Then, 10-fold dilutions were prepared up to 10^{-6}, and these dilutions were spread onto PSM in triplicate. The plates were incubated for 3-7 days at 28ºC and checked to determine if there were hydrolysis halos around the colonies. Next, the proportion value (D/d) between the halo diameter (D) and the fungal colony diameter (d) was recorded. Finally, the screened fungal colony with obvious halos was purified and inoculated onto potato glucose medium and then preserved at 4ºC.

Second Screening: The following constituted the fungus second screening medium ($g \cdot L^{-1}$): KH_2PO_4 2, $(NH_4)_2SO_4$ 2.5, $MgSO_4 \cdot 7H_2O$ 0.3, $CaCl_2 \cdot 2H_2O$ 0.3, $FeSO_4 \cdot 7H_2O$ 0.005, $MnSO_4 \cdot H_2O$ 0.0016, $ZnSO_4 \cdot 7H_2O$ 0.002, $CoCl_2 \cdot 6H_2O$ 0.0017, Tween-80 1 mL, CMC-Na 20, and a natural pH value. The second screening process was based on the cellulose activity. The selected fungal strain was inoculated in PSM onto the fungus second screening medium according to the principle of one cycle spore for one shaken flask. There was 50mL of medium in a 250mL triangular flask, which was stored at 30ºC with 200rpm shaking. This was repeated three times, and after 5 days of fermenting, the cellulose activity was measured.

Fungus Filter Paper, Straw Disintegration Test: The following constitutes the fungus filter paper, straw disintegration test medium ($g \cdot L^{-1}$) ： filter 2.0 or straw 2.0, $(NH_4)_2SO_4$ 3.0, KH_2PO_4 2.0, $MgSO_4 \cdot 7H_2O$ 0.4, and a natural pH. A fungus spore suspension with 5% inoculum size ($1 \times 10^8 \cdot mL^{-1}$) was inoculated into a triangular flask containing the liquid nutrient medium (the medium was 1/5 of the flask's volume). The flask was incubated at 30ºC and was hand-shaken 2 or 3 times daily when it was observed every day. The water content of initial Straw powder was 10.2%in straw disintegration test.

Cellulose Measuring Methods (Qi *et al*., 2004)

CMCase: First, 1.5 mL of substrate (1% CMC-Na solution) was added to 0.5 mL of appropriately diluted enzyme liquid. The mixture was incubated in a 50ºC water bath for 5 min. Next, 1.5 mL of 3,5-dinitrosalicylic acid was added, and the mixture was incubated at 100ºC for 5 min. After incubation, the mixture was immediately cooled in tap water. Then, distilled water was added to a constant volume of 10 mL. The mixture was shaken, and the reducing sugar content was determined.

FPA: Fifty mg filter paper (1×6 cm) slip was weighted as substrate, and then, small rolls were set and inserted into the bottom of a test tube (1.2cm×18cm). Then, 0.5 mL of diluted enzyme liquid and 1.5 mL of acetic acid-sodium acetate buffer solution (pH 4.8) was added, followed by incubation in a 50ºC water bath for 1 hour. Next, 1.5 mL of DNS was added, followed by incubation at 100ºC for 5 min. After the incubation, it was immediately cooled in tap water. Finally, distilled water was added to a constant volume of 10 mL. It was shaken, and the reducing sugar content was determined.

For the aforementioned assays, an inactivated crude enzyme solution that had been incubated at 100ºC for 5 min was used as a blank control and the process was repeated three times. Enzyme activity was expressed as international unit (IU). One enzyme activity unit is defined as the enzyme quantity needed by 1mL of cellulose enzyme liquid to catalyze substrate into 1μmolof glucose in 1 minute, and it is represented as $IU \cdot mL^{-1}$.

Optimized Conditions of Fungus for Producing Enzymes:

The following procedure was carried out with reference to the methods of Yang and other researchers (2010). The following constituted the basal liquid fermentation medium ($g \cdot L^{-1}$): KH_2PO_4 2, $(NH_4)_2SO_4$ 2.0, $MgSO_4 \cdot 7H_2O$ 0.3, $CaCl_2 \cdot 2H_2O$ 0.3, $FeSO_4 \cdot 7H_2O$ 0.005, $MnSO_4 \cdot H_2O$ 0.0016, $ZnSO_4 \cdot 7H_2O$ 0.002, $CoCl_2 \cdot 6H_2O$ 0.0017, Tween-80 1 mL, CMC-Na 20, and a natural pH.

First, each of the following was used as a substitute for the carbon source in the basal medium: 2% straw powder (SP), bran (BR), microcrystalline cellulose (MCC), straw powder + bran (SP＋BR), straw powder + microcrystalline cellulose (SP ＋ MCC), and bran +

microcrystalline cellulose (BR + MCC). All other ingredients and quantities remained the same. For each of the experimental carbon sources, 50 mL of the appropriate basal medium was placed into a 250 mL triangular flask, which was then inoculated with a spore suspension with a 5% inoculum size. The culture was incubated at 30 ºC with 200 rpm shaking for 120 h. Then, both the CMCase and FPA activities were measured, and the best carbon source was determined.

The optimal carbon source was used as the carbon source for the following optimization experiments. Next, five different compound ratios (5:5, 6:4, 7:3, 8:2 and 9:1) of the carbon source were selected to carry out the fermentation culture, keeping all other fermentation conditions unchanged. Also, five concentrations (1%, 2%, 3%, 4%, and 5%) of carbon source were used to experimentally determine the optimal carbon source concentration for producing enzymes. Both aspects were determined according to the CMCase and FPA activities.

Next, the optimal nitrogen source was determined experimentally. The following represents the candidates for the best nitrogen source: 2% urea, peptone (PP), potassium nitrate (PN), ammonium nitrate (AN), ammonium chloride (AC), and ammonium sulfate (AS). Each of the aforementioned sources was experimentally used as the single nitrogen source, and the best nitrogen source was determined by measuring the CMCase and FPA activities. Additionally, six different concentrations (0.2%, 0.3%, 0.4%, 0.5%, 0.6%, and 0.7%) of nitrogen source were tested in order to determine the optimal nitrogen concentration for producing enzymes. Again, the CMCase and FPA activities were assessed in order to determine the optimal concentration.

After determining the best carbon and nitrogen source conditions, we also determined the optimal pH, inoculum size, incubation time, and incubation temperature. First, the initial fermentation medium pH was respectively adjusted to 3, 4, 5, 6, 7, and 8, and then, the degradation of cellulose (CMCase and FPA) was measured in order to determine the most suitable pH for producing enzymes. Based on the above research, different inoculum sizes (2%, 3%, 4%, 5%, 6%, 7%, and 8%) of the F12 spore suspension was used to carry out the same experiment, and the CMCase and FPA activities were measured to determine the most suitable inoculum size. Next, using these optimized culture conditions, the CMCase and FPA activities of F12 were measured after 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h, and 192 h to determine the peak hours of enzyme production. Finally, again using the optimized conditions, various temperatures (25 ºC, 35 ºC, 40 ºC, and 45 ºC) of the strain F12 were analyzed, and the CMCase and FPA activities were measured in order to determine the optimal temperature for enzyme production.

Identification of Fungus: Colony Morphology and Microscopy Morphology Observation: The fungal strains were inoculated on the potato glucose medium, and the colony morphological characteristics were observed after cultivation for 5 days at 28ºC. A few spores and hypha flakes were selected. The morphological characteristics as well as the insertion style of the spore and sporophore were observed with a LE01530 scanning electron microscope.

Total DNA Extraction, 18S rDNA Amplification, and Sequencing: The genome DNA of the isolated strain was extracted using a microorganism genome extraction kit (American MP Bio medicals Company). Using the extracted DNA as the template, primers p1(5'-TCCGTAGGTGAACCTGCCG-3') and p2 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al*., 1990) were used to carry out the amplification of the 18S rDNA gene (American BioRad Company, PTC200 thermal cycler). The reaction conditions were the following: 95ºC pre-denaturation step for 5 min, 35 cycles of: 95ºC denaturation step for 40 s, 55ºC annealing step for 60 s, 72ºC extension step for 120 s, and a 72ºC extension step for 10 min. Then, 1% agarose gel electrophoresis was used to detect the PCR products. At a certain ratio, the PCR products were combined with the pMD 18-T carrier. The following is the composition of the ligation system: pMD18-T carrier 1 µL (about 50 ng), PCR products 4 µL (about 300 ng), ligase buffer 1 µL, and T4 DNA ligase (5 U/µL) 1 µL. The ligation system was incubated at 16ºC for 12~16 h. Then, the ligation was electro-transformed into competent *E. coli* DH5α cells, and the transformed fungal liquid was spread onto LB agar that consisted of 24 µg/mL of Isopropyl-*β*-D-thiogalactopyranoside (IPTG), 40 µg/mL of 5-bromo-4-chloro-3-indolyl-*β*-D-galactoside (X-gal), 50 µg/mL of ampicillin (Amp). The plates were incubated overnight at 37ºC. The blue and white colonies were screened, and a white colony was selected from one of the plates. The selected colony was sent to the Dalian TaKaRa Biotechnology Company for sequencing, and the obtained sequence was analyzed with DNAMAN software.

Construction and Analysis of Phylogenetic Tree: The homology analysis of the 18Sr DNA was carried out by using Blast software. An evolutionary tree was conducted by using the Neighbour-Joining method (using 1000 bootstrap replicates) in the PHYLIP software (version 3.572).

Analog Experiments of Compost Dehydration Effects: First, 60 g of dried straw powder was passed through a 60-mesh sieve and placed into a 250mL erlenmeyer flask. Gradually, 230mL of 0.6% ammonium sulfate solution was added to the flask, and then, the mixture was mixed with a glass rod. At this time, the water-holding capacity of the material reaches the maximized value, and no

water separates out naturally from the material. The material was then sterilized at 121.3 ºC for 30 min. After sterilization, the material was allowed to cool. Then, 10 mL of the 1×10^8 mL^{-1} spore suspension of *Penicillium oxalicum* F12 was placed into the triangular flask for the analog experiments of compost dehydration effects. To serve as an experiment control, 10 mL of sterile distilled water was placed into a separate triangular flask. In order to ensure that the material was uniformly distributed, a sterile glass rod was used for mixing. Each was processed three times. Then, the flasks were incubated at 30ºC, and the flasks were shaken twice a day. The culture continued for 10 days.

RESUITS

Isolation and Screening of Fibrinolytic Fungus: A fungus called F12 was isolated by using the comparatively large halo produced on the cellulose—Congo red isolation medium. After 5d of shaking flask fermentation in the second screening medium, the CMCase was 27.50 IU·mL^{-1}, and the enzyme activity of FPA was 6.10 IU·mL^{-1}. In the filter paper and straw disintegration test, the margin of the filter paper began to ulcerate the third day after inoculation; floccules occurred on the surface of the filter paper, and the filter paper began to soften. On the fourth day, the filter paper began to disintegrate, and a large amount of floccules occurred in the solution. By the tenth day of the culture, all of the filter paper strips had disintegrated into fragments. Five days after culturing, the straws began to soften, and after 17 days, the straws become small and loose.

Optimized Conditions of Fungal Culture for Producing Enzymes: The relationship among the cell growth and the CMCase and FPA activities was determined. The CMCase and FPA activities were positively related to the cell growth (Fig. 1). The maximum CMCase and FPA activities were nearly achieved at the maximum mycelial weight. The optimal incubation time was 120h (Fig. 1). In addition, the most suitable carbon source for *P. oxalicum*F12 was 2% of bran and microcrystalline cellulose mix (6:4) (Fig.2a-c). Moreover, the optimal nitrogen source was ammonium sulfate (0.2%) (Fig.2d-e), and the most suitable pH was 5 ～6 (Fig.2f). The optimal inoculum size was 5% (Fig. 2g), and the optimal temperature was 30～35ºC (Fig. 2h). Under these conditions, the CMCase of this fungal strain achieved 47.50 IU·mL^{-1}, and the FPA achieved 11.10 IU·mL^{-1} (Fig. 2).

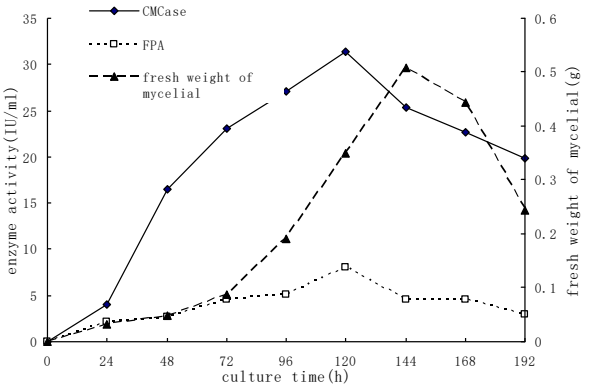

Fig.1.Change in the F12CMCase, FPA, and fresh weight of mycelial at different culture times

Identification of F12 isolate Colony Morphology and Microscopy Morphology: The fungal colony was grayish-green, was comparatively thick, and had a fluffy texture and white-hair edge; the reverse side was pale brown, and the diameter of the 3～4d fungal colony reached 2～3 cm. The hypha is septa hypha, and a lot of conidial structures formed. Furthermore, the conidiophore wall is smooth and colorless, and most of them are double wheel and compact at 140-200×3.5-4.0μm. The phialide is columnar with a short neck at 9.2-13×2.5-3μm. Additionally, the conidium is oval and it has a smooth wall. The conidium arranged in clusters at 4.5-5.3×2.5-3μm. The spore easily drops (Fig. 3). Based on *Microbial Taxonomy* (Zhang, 1990), the fungal strain F12 was preliminary judged as belonging to the *Penicillium* sp.

Molecular Biology Identification: By using the total extracted DNA of the cell as template and by employing primers P1 and P2, we amplified the 18S rDNA gene of strain F12. A fragment of approximately 0.6 kb was obtained through PCR amplification (Fig. 4). After sequencing, it was determined that the actual length of this fragment was 553 bp. (GenBank Accession number KF997090.). The similarity analysis of this sequence and the relevant data in Genbank revealed that its homology (homology, 100 %/553 bps, based on 18S rDNA) with *Penicillium oxalicum* (HM053477) is the highest. By comparing this with the already known *P. oxalicum* 18S rDNA reported by NCBI, we used PHYLIP (version 3.572) software to construct an evolutionary tree as well as determine the evolutionary stage of the fungal strain (Fig. 5). Through a combination of physiology, biochemistry, and molecular identification, we determined that this fungal strain was *Penicillium oxalicum*, and it could be named *Penicillium oxalicum* F12

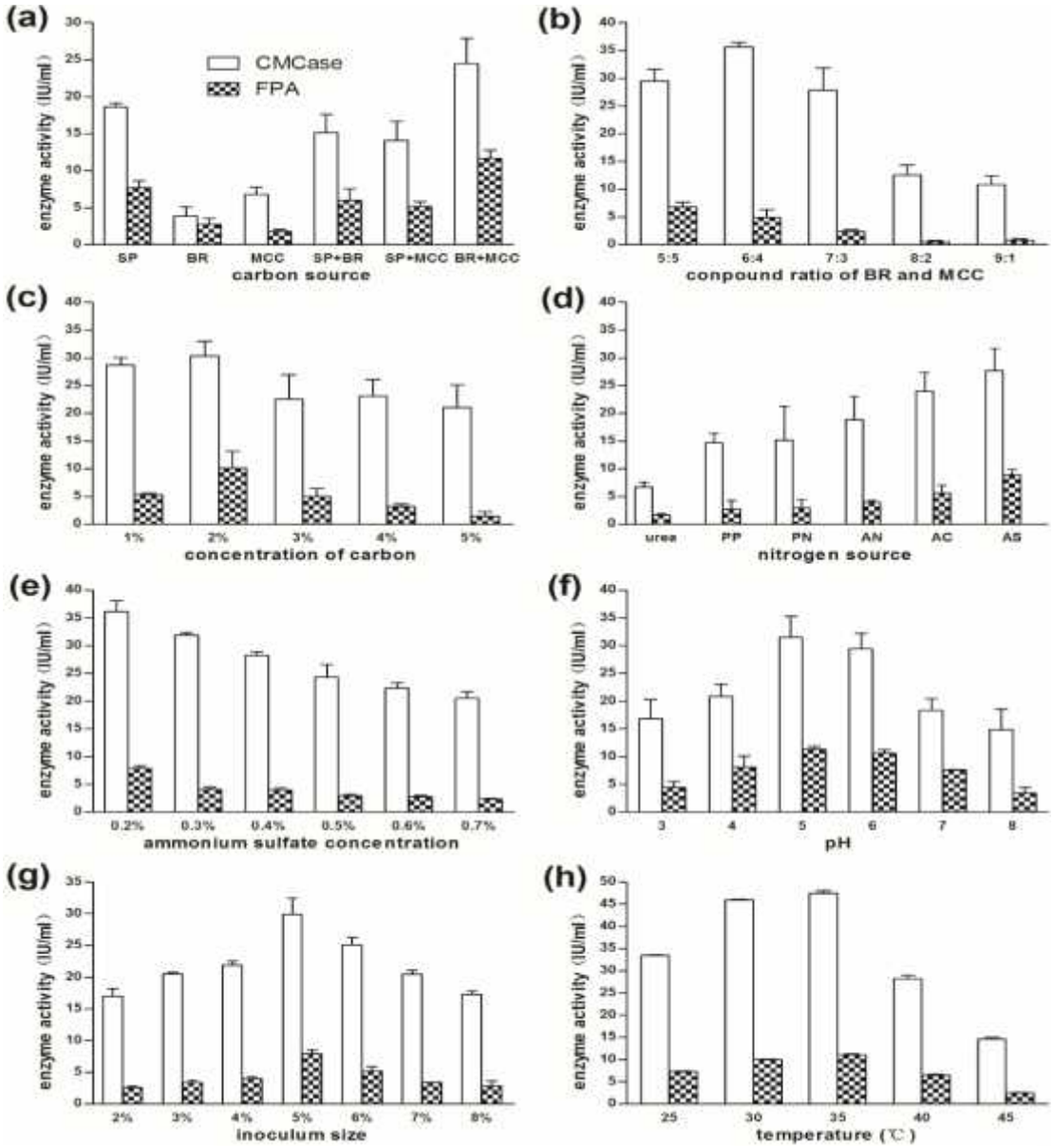

Fig.2. Screening and optimization of culture and enzyme production conditions of isolate F12: (a) carbon source, (b) compound ratio of BR + MCC,(c) concentration of carbon source, (d) nitrogen source, (e) concentration of nitrogen source, (f) pH, (g) inoculation quantity, and (h) cultivation temperature.

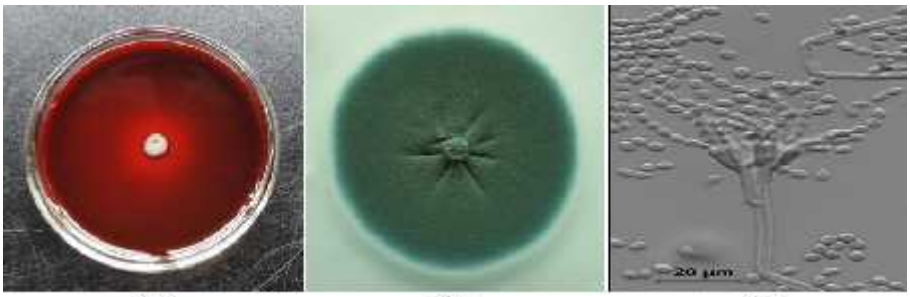

Fig.3. Cultural characteristics of strain F12 on (a) cellulose - Congo red medium, (b)PDA medium, and (c)the morphological characteristics and the strain F12 spores and spore stems.

Fig. 4.Agarose gel electrophoresis of the resulting PCR fragments of the 18S rDNA (1) DNA Marker, (2) F1218S rDNA, and (3) F12 18S rDNA.

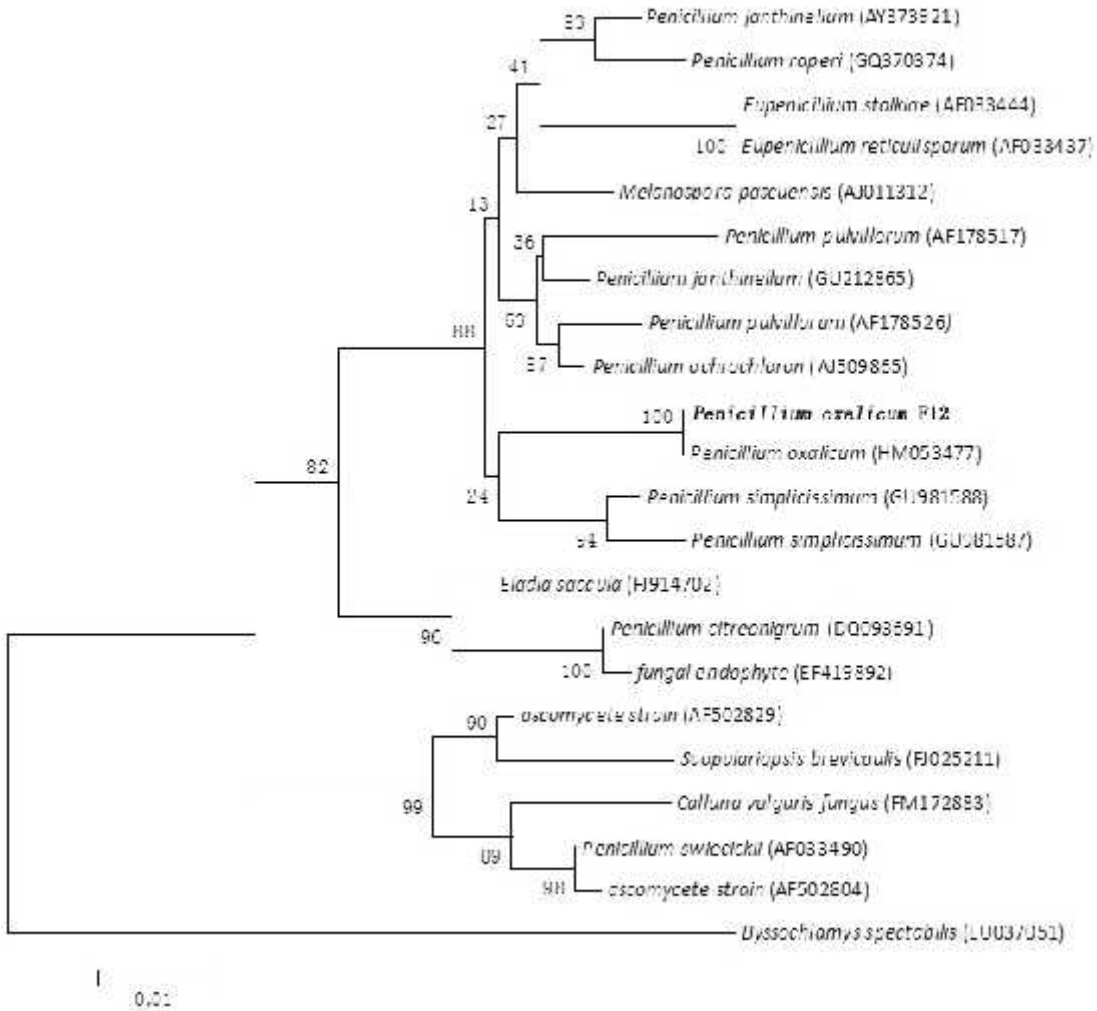

Fig. 5.Phylogenetic dendrogram for strain F12 and related strains based on the 18S rDNA sequences. The details given in parentheses are the accession numbers of published sequences. The Neighbour-Joining method with bootstrap values of 1000 iterations was used.

Analog Experiments of Compost Dehydration Effects: Straw powder inoculated with *Penicillium oxalicum* F12 was incubated at 30ºC for 10 days (Fig. 6). It could be seen that water separated out gradually from the material,

but the control material did not exhibit any change. After filtering with double layers of gauze, the material inoculated with the fungus filtered out the water. However, the control material did not filter out any water, except the 8.3g of water absorbed by the gauze (Fig. 6 and Table 1). Additionally, after the filter residue was centrifuged at 4200 rpm for 20 min, the material that was inoculated with the F12 isolate lost 28.0g of water, but the control material did not lose any water (Table 1). Therefore, it was clear that the inoculation with *P. oxalicum* F12 considerably changed the water storage structure of the analog compost material so that the absorbed water of the straw powder changed into bulk water and flowed out. The initial water ratio of the inoculated F12 material filter residues was reduced from 80% to 75.3%, and the initial water ratio of the control material filter residues was reduced from 80% to 79.2% (Table 2). Therefore, the water ratio of the inoculated analog compost material was reduced by 3.9%, and the dehydration volume reached 47.6g, which amounted to 19.8% of the initial material water contents as compared with the control material.

Fig.6.Dehydration effect of simulated composting

Table 1. The filtration and centrifugal water in different simulated composting treatments

	Control				Treatment			
	Ⅰ	Ⅱ	Ⅲ	Average	Ⅰ	Ⅱ	Ⅲ	Average
Water in cotton gauze, （g）	8.3	7.7	8.9	8.3	9.6	9.0	8.9	9.3
Filtration water, （g）	0.0	0.0	0.0	0.0	25.9	13.7	16.1	18.6
Centrifugal water, （g）	0.0	0.0	0.0	0.0	20.2	28.7	35.2	28.0
Total water, （g）	8.3	7.7	8.9	8.3	55.7	51.4	60.2	55.9

Table 2. Comparison of bio-dehydration in different simulated compost treatments

	Initial moisture content of the material	moisture content of residue after dehydration （%）	P ≤0.05	P ≤0.01
Control	80	79.2	a	A
Treatment	80	75.3	b	B

DISCUSSION

The proportion of cellulose material in the excrement produced by livestock and poultry is relatively large. Due to the water-insoluble high crystal structure of cellulose and the surrounded xylogen layer, celluloses can hardly be effectively degraded; therefore, this influences the whole composting process of organic wastes to a certain degree. Specially, the great amount of cellulose and hemicellulose substances in excrement produced by livestock and poultry (which reaches 40% and 27% respectively (Ling *et al*., 2008) as well as the compact hole structure formed by a great deal of cellulose both cause the high water ratio of excrement produced by livestock and poultry to be relatively large.

It also has the protection of colloid material so that the water is hard to volatilize, thereby causing a slow decay speed and other specific characteristics. Furthermore, the actual research work conducted on the manual decomposition of cow manure is relatively scarce (He *et al*., 2006).In this study, a fungus was isolated from cow dung and cow dung natural composts samples and was identified as *Penicillium oxalicum*. Under suitable conditions for producing enzymes, the average CMCase was 47.50 IU·mL^{-1}, and the average FPA was 11.10 IU·mL^{-1}. These enzyme activities were equivalent to *P. oxalicum* F67 that was isolated from the goose intestinal tract by Zhang *et al.* (2009). Furthermore, *P. oxalicum*, as a functional fungal strain, has been researched and reported in terms of agriculture, fodder, and wastewater disposal (Fan *et al*., 2002，Peng *et al*., 2004，Yue *et al*., 2008，Zheng *et al*., 2008), but it has rarely been reported in terms of animal manure compost disposal.

The experimental results of the analog compost dehydration effects revealed that the *P. oxalicum* F12 fungal strain can grow rapidly in very wet material. Compared to the control material, the water content of the compost material residue inoculated with the fungus was reduced by 3.9%. The average water content was 47.6g, which accounted for 19.8% of the initial material water content. Perhaps, the *P. oxalicum*F12 fungal strain effectively degraded the fiber material and changed the structure, which then transformed the absorbed water into bulk water so that it could flow out. This offers a convenient solution to the rapid decomposition of very wet material.

This paper makes use of straw powder and the analog excrement produced by livestock and poultry, which is very wet and high in cellulose content, to investigate the *in situ* biological dehydration effects of *P. oxalicum* F12. Moreover, *P. oxalicum* belongs to the fungi subdivision Deuteromycotina, which has relatively high cellulose enzyme activity and can also secrete pectinases. Furthermore, it affects the compact structure that is formed by cellulose and colloid in both pig and cow manure (Wang. *et al*., 2010a). In addition, the optimal temperature of *P. oxalicum* F12 for producing enzymes (35 ºC, Fig.2) also meets the requirement of the initial compost stage. However, due to the diversity and the complexity of ingredients in actual compost material as well as the presence of a large number of indigenous microorganisms, the real effects of its application in actual composts remain to be further analyzed.

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REFERENCES

Adsul, M. G., K. B. Bastawde, A. J. Varma and D. V. Gokhale (2007). Strain improvement of Penicillium janthinellum NCIM1171 for increased cellulase production. Bioresource Technology, 98(7): 1467-1473.

Chang Z.Z., X.M. Ye, H.Y. Huang and H.Y. Fei (2007). Improvement of Cow Excreta Dewatering Characteristic with Inoculation of Fungus. J. Agri-Environ. Sci., 26(6):2345–2349.

Fei H.Y., Z.Z. Chang, S.M. Wang, H.H. Huang, X. Chen and H. Zhu (2006). Characterization of Moisture in Three Livestock Manures. J. Agri-Environ Sci., 25 (Suppl.):599–-603.

Fan B.Q., J.Y. Jin and C. Ge (2002). Isolation of *Penicillium oxalicum* and Its Effect on Solubilization of Insoluble Phosphate under Different Conditions, Scientia Agri. Sinica, 35(5):525-530.

He L.Y., G.X. Cao, X.F. Sheng, Y.L. Qian and L. Liu (2006). Effects of Decomposing Microbial Inoculums NMF on Quick Decomposition of Cow-Dung. Chinese J of Soil Sci., 37(4):761-763.

Huang H.Y., W.B. Zhu, Z.Z. Chang, Y. Ma, and X. M. Ye (2003). Effect of composting methods on fermentation and dehydration of cow and poultry manures. Rural Eco-Environ., 19(1):53-55.

Ling J., J. Li, M.C. Liu, F.L, Yan, J.J. Xu, L.H. Lu and D. Yan (2008). A New Thought to Controlling the Pollution of Livestock Excrements. J of Sichuan Forestry Sci. and Tech., 29(4):99-102.

Peng X.W., H.X. Zhang and Z.H. Bai (2004). Induced resistance to Cladosporium cucumerinum in cucumber by pectinases extract of *Penicillium oxalicum*, Acta Phytopathologica Sinica, 34(1):69-74.

Qi X.J., J.X. Gou, X.J. Han and B. Yan (2004). Study on Measuring Reducing Sugar by DNS Reagent. J Cellulose Sci. and Tech. 12(3):17-30.

Sun X.Y., Y.B. Qu and Z.Y. Liu (2007). Progress in Research of Lignocellulose-degrading Enzymes from Penicillium. Chinese J of Applied and Environ. Bio., 13(5):736-740.

Wang B.W., X.X. Jiang, P. Sun and N. Wang (2010a). Study on the Effects of *Penicillium oxalicum* Currie & Thom Producing Pectinase on Digestive Physiology of Broilers, Chinese J of Anim. Nutrition, 22(2):358-364.

Wang F.H, W.Q. Ma, Z.X. Dou , L. Ma, X.L. Liu, J.X. Xu, and F.S. Zhang (2006a). The estimation of the production amount of animal manure and its environmental effect in China. China Environ.

Sci., 26(5):614-617.

Wang, Z. C., W. J. Lu and H. T. Wang (2006b). Research on the Isolation and Characters of Several Strains of Thermophilic Cellulolytic Fungus. Acta Scientiarum Naturalium Univ. Pekinensis, 42(2):259-263.

Wang H.Y. and B.Q. Fan (2010b). Screening of three straw-cellulose degrading microorganism. Acta Microbio. Sinica, 50(7):870-875.

White, T. J, T.Bruns and S. Lee (1990).PCR Protocols: A Guide to Methods and Applications [M]. New York: New York Academic Press Inc., 315-322.

Yang Y.K., F.X. Zhu, W.P. Wang, X.Y. Chen, C.L. Hong, C.Z. Wu and Z.Y. Xue (2010). Screening of the cellulase-producing fungi and its enzyme-producing conditions in compost. Acta Agriculturae Zhejiangensis, 22(4):464-468.

Yue B., B.W. Wang, M.G. Zhang, L.Z. Jing and Q. Zhang (2008). The effect of *Penicillium oxalicum* Currie & Thom from Goose on Solubilizing Phosphate and on Phosphorus Metabolism for Goose's, J of Qingdao Agri. Univ. (Natural Science), 25 (1):34-37.

Zhang J.Z. (1990). Microbial Taxonomy. Shanghai: Fudan Univ. press.

Zhang Q., B.W. Wang, M.G. Zhang, X.X. Jiang, N. Wang, Q.L. Wang and Y.C. Fan (2009). Ferment Conditons of Goose Origin *Penicillium oxalicum* Currie & Thom F67 Producing Cellulase. J of Shenyang Agri. Univ., 40(1):47-52.

Zheng W.C., B.P. Xin, Y.L. Gan and C.P. Li (2008). Studies on *Penicillium oxalicum* growing mycelium sorption to complex-Cu reactive dyes, China Environ. Sci., 28(2):142-146.

Zhang Y.H., J. Wang, W. Zhang, C.W Li,, L. Ma and W. Zhou (2004). An Improved Differential Medium for Cellulose Decomposing Microorganisms. J Cellulose Sci. and Tech. 12(1):33-35.