SCREENING OF *CORDYCEPS* STRAINS AND OPTIMIZATION OF ITS SOLID-STATE FERMENTATION CONDITIONS ON BIOCONVERSION OF *ASTRAGALUS* RESIDUE

ZHEN-YUAN ZHU,[*] YANG LI,[*] HUI-QING SUN,[*] LI-JING CHEN,[**] YA-LI TANG,[*] XIAO-CUI LIU[*] and YONG-MIN ZHANG[***]

[*]*Key Laboratory of Food Nutrition and Safety, Ministry of Education, College of Food Science and Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, P.R. China*
[**]*Key Laboratory of Freshwater Fishery Germplasm Resources, Ministry of Agriculture, Shanghai Ocean University, 200090, P.R. China*
[***]*Pierre and Marie Curie University-Paris 6, Paris Institute of Molecular Chemistry, UMR CNRS 8232, 4 Place Jussieu, 75005, Paris, France*
✉ *Corresponding author: Z.-Y. Zhu, zhyuanzhu@tust.edu.cn*

Received February 25, 2014

The *Astragalus* residue is produced after the extraction of *Astragalus* polysaccharides (APS). In *Astragalus* residue, the main ingredient is crude fiber. The crude fiber is considered to be the main source for green chemicals, bio-fuels and bio-based products. In this study, we used five kinds of *Cordyceps* as fermentation strains and *Astragalus* residue as fermentation substrate for solid-state fermentation. Crude fiber degradation rate after fermentation was considered as the main indicator. The mannitol content and soluble sugar content of the fermentation product were used as secondary indicators. The experiments were carried out by screening the solid-state fermentation strains, optimizing the solid-state fermentation conditions and studying the pilot-scale cultivation of solid-state fermentation. From the results of screening the solid-state fermentation strains, we selected the *Paecilomyces bainier* strain for further investigation. The fermentation conditions were optimized with four single factors, including fermentation time, solid fermentation inoculum size, pH value of solid medium and fermentation substrate thickness. The single-factor tests revealed that the optimum conditions were the following: fermentation time of 25 days, fermentation substrate pH of the initial value, inoculum of 20% and fermentation substrate thickness of 1.0 cm.

Keywords: *Astragalus* residue,·*Cordyceps Paecilomyces sinensis*,·solid-state fermentation

INTRODUCTION

Astragalus is the root of *Astragalus mongolicus* and *Astragalus membranacus*, which are leguminous plants. The *Astragalus* contains large amounts of flavones, alkaloids, polysaccharides, saponins, and other active ingredients.[1] Studies showed that *Astragalus* polysaccharides (APS) can improve animal performance, enhance immune function and disease resistance, and exhibit many other functions.[2,3] In industrial production, most of the methods used to extract APS still rely on water extraction and alcohol precipitation. The yields of APS are generally not satisfactory with these methods. Furthermore, these methods produced large numbers of residue and allowed other pharmaceutical ingredients of *Astragalus* remain in the residue. A study investigating the influence of *Astragalus* residue on white ducks' production performance and immune function was reported.[4] It proved that the *Astragalus* residue promoted the white ducks' digestion ability and increased the feed concentration. Thereby, it increased the body weight and the weight of the ducks' immune organs, as well as improved their immunity. After removing the active ingredients from *Astragalus* residue, the

main remaining ingredient is crude fiber, which is considered to be the main source for green chemicals, bio-fuels and bio-based products. Waste would be created without an appropriate approach to deal with the residue. Thus, it is of great importance to effectively reuse the residue of *Astragalus.*

Cordyceps is a rare traditional tonic herbal medicine. It contains *Cordyceps* polypeptide, polysaccharide, sterol, enzyme, adenosine and other active substances.[5] Its physiological functions are extremely rich.[6,7] *Cordyceps* plays an important role in cellular immunity and humoral immunity. It can improve the phagocytosis of monocyte macrophage, promote the fatigue recovery and inhibit the growth of tumour. In China, the products of *Cordyceps* are mainly used in health industry. *Cordyceps* is also an effective animal feed additive. It can stimulate the growth of piglets and reduce the fodder-meat rate. Improving the total content of protein and amino acids of broiler chicken breast and leg muscle is another function of *Cordyceps*.[8,9,10] It has been reported that the *Cordyceps sinensis* works best on broiler chickens' average daily gain and the fodder-meat rate and promotes the role of cellular and humoral immunity of broiler chickens.[11] Wild *Cordyceps sinensis* resources are very rare. After several years of research and development, scientists have established a method of artificial solid *Cordyceps* culture, named *Cordyceps* fungi fermentation. In this process, after harvesting the fruiting bodies, the culture medium is covered with mycelia of *Cordyceps*. It is called *Cordyceps* culture medium (CCM). Meng Cuiliang[12] found that the Cordyceps culture medium has a great influence on the growth performance and immune function of broiler chickens. Dai Chaozhou[13] studied the influence of *Cordyceps* culture medium on the growing-finishing pig performance and carcass quality. Both of their investigations showed that the *Cordyceps* culture medium could promote animal growth and improve the immune system function.

Several reviews on solid-state fermentation (SSF) have already been reported.[14,15,16,17] They considered the general aspects of SSF and its application to the development of several bioprocesses. SSF is defined as a fermentation process occurring in the absence of free flowing water and employing either a natural support or an inert support as a solid material.[18] The wide range of solid materials used in SSF can be classified into two categories: inert materials, which only act as an attachment place for the microorganism, and non-inert materials, which not only function as an attachment place, but also supply some nutrients to the microorganism.[19] The latter, due to its double role, is also named support-substrate. These materials are typically starch- or (ligno-) cellulose-based agricultural products or agro-industrial sources, such as potato peel,[20] or corn residues.[21,22,23] Studies on soy residue[24] and wheat straw[25] solid-state fermentation of apple pomace fermentation technology have also been reported.[26] In addition, the utilization of this kind of support helps in solving both the economic and the environmental problems caused by their disposal.

In this study, we used *Cordyceps* as fermentation strain and *Astragalus* residue as fermentation substrate for solid-state fermentation. The crude fiber degradation rate after fermentation served as the main indicator. The mannitol content and the soluble sugar content of fermentation products were used as secondary indicators. The experiments were carried out by screening the strain of the solid fermentation, optimizing the solid-state fermentation conditions and studying the pilot-scale cultivation of solid-state fermentation.

EXPERIMENTAL

Astragalus residue

The *Astragalus* residue was produced after the extraction of *Astragalus* polysaccharides. It was determined that the residue had the following composition: 42.74% crude fiber, 1.11% mannitol, 7.04% soluble saccharide, 3.81% reducing sugar, 0.044% adenosine, 0.031% cordycepin, 0.48% fat and 8.92% protein.

Microorganisms

Five kinds of *Cordyceps* strains were used: *Paecilomyces bainier* (DCXC), *Cordyceps gunnii* (ZZY), *Cordyceps cicada* (ZZYC), *Cordyceps militaris* 1 (ZZYYS), *Cordyceps militaris* 2 (ZZYZB). All strains came from the Biological Resources and Functional Foods Research of Tianjin University of Science and Technology.

Medium culture

A potato-dextrose-agar medium (PDA medium) was used as strain activation medium. The composition of the medium was the following: potato 20%, glucose 2% and agar 1.5%-2.0%, with initial pH value.

The liquid seed medium was composed of sucrose 2%, peptone 1%, potassium dihydrogen phosphate (KH_2PO_4) 0.3%, magnesium sulfate ($MgSO_4$) 0.15%,

with initial pH value.

The solid-state fermentation medium contained 100 g *Astragalus* residue, 4.00 g glucose, 1.00 g peptone and 150 mL distilled water.

All media were autoclaved for 20-30 min, at a pressure of 0.1 MP and temperature of 121 °C.

Strain screening

The activation of slope strain: The five strains were put on PDA slant medium and stored at 4 °C in the refrigerator. The PDA tubes slant culture method was used to activate the five strains. After the cultivation, these strains could serve as fermentation liquid seed parent species to be used for subsequent experiments.

Preparation of seed solution: 100 mL of the liquid seed medium was placed in a 250 mL Erlenmeyer flask and sterilized as liquid seed medium. By using an inoculating needle, 0.25 cm^2 of slope strains was inoculated in the liquid seed media on the ultra-clean workbench. The medium was placed in a thermostat shaker at 20 °C and 160 rpm, and cultured for 3 days in the dark. They were used as seed in the experiments that followed.

Solid-state fermentation: The seed solution was inoculated in the solid-state fermentation medium with 20% of the inoculum size. It was then incubated at 20 °C for 35 days in the dark.

Post-fermentation processing: The fermentation product was dried to constant weight at 65 °C, weighed and smashed. The crude fiber content, mannitol content and the soluble sugar content in the fermented products were determined.

Optimization of fermentation conditions

From the results of the strain screening experiment, the *Paecilomyces bainier* (DCXC) was selected as fermentation strain. Then the fermentation conditions were optimized with four single factors: fermentation time, solid fermentation inoculum size, pH value of solid medium and fermentation substrate thickness.

Preparation of seed solution: 100 mL of liquid seed medium was placed in a 250 mL Erlenmeyer flask and sterilized. By using an inoculating needle, 0.25 cm^2 of *Paecilomyces bainier* (DCXC) slope strain was inoculated in the liquid seed medium on the ultra-clean workbench. The medium was placed in a thermostat shaker at 20 °C and 160 rpm and cultured for 3 days in the dark. It could be used as seed in the experiments that followed.

Single factor test: fermentation time

The seed was inoculated to the solid fermentation medium with 20% inoculum size on the ultra-clean workbench and cultured at 20 °C in the dark. The pH value was the initial one and the thickness of the solid state fermentation medium was 1.0 cm. Fermentation times were 0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 days. The fermentation products were dried to constant weight at 65 °C, weighed and smashed. Then the crude fiber content, mannitol content and soluble sugar content in the fermented products were determined.

Single factor test: inoculum size

The seed was inoculated to the solid fermentation medium on the ultra-clean workbench and cultured at 20 °C in the dark. The pH value and thickness of the solid state fermentation medium were initial value and 1.0 cm, respectively. The fermentation time was 35 d. The inoculum sizes were 10%, 20%, 30%, 40%, 50%, and 60%. The fermentation products were dried to constant weight at 65 °C, weighed and smashed. Then the crude fiber content, mannitol content and soluble sugar content in the fermented products were determined.

Single factor test: pH of fermentation medium

The seed was inoculated to the solid fermentation medium with 20% inoculum size on the ultra-clean workbench and cultured at 20 °C in the dark. The thickness of solid-state fermentation medium and fermentation time were 1.0 cm and 35 days, respectively. The pH values of the media were 1.0, 2.5, 4.0, initial pH value, 5.5, 7.0, 8.5 and 10.0, respectively. The fermentation products were dried to constant weight at 65 °C, weighed and smashed. Then the crude fiber content, the mannitol content and the soluble sugar content in the fermented products were determined.

Single factor test: fermentation substrate thickness

The seed was inoculated to the solid fermentation medium with 20% inoculum size on the ultra-clean workbench and cultured at 20 °C in the dark. The pH value of the solid-state fermentation medium was the initial one. The fermentation time was 35 days. The thickness of the fermentation substrates was 0.5 cm, 1.0 cm, 1.5 cm, 2.0 cm, 2.5 cm or 3.0 cm. The fermentation products were dried to constant weight at 65 °C, weighed and smashed. Then the crude fiber content, mannitol content and soluble sugar content in the fermented products were determined.

Pilot scale cultivation of solid fermentation

For the pilot scale cultivation experiment, we adopted new platter fermentation. The new platter was rectangular with the following size: 280 mm × 180 mm × 120 mm. The new platter was made of polypropylene and had a sterile air vent on one side. At the beginning of fermentation, the *Astragalus* residue was put on the platter. Then the platter was placed in a plastic bag made of high temperature resistant polypropylene material. A tampon was used to seal the vent. Then the sterilization, inoculation, and fermentation were carried out.

Preparation of solid-state fermentation medium: 200

g of *Astragalus* residue was put into the new platter. The nutrient solution contained 8.0 g glucose, 2.0 g protein peptone and 300 mL distilled water. The *Astragalus* residue was mixed with the nutrient solution in a ratio of 1:1.5 and stirred well. The pH value of the solid-state fermentation medium was the initial one.

The new platter was placed in a plastic bag made of high temperature resistant polypropylene material. A tampon was used to seal the vent. More media were prepared in this way. The media were autoclaved for 20 min-30 min at a pressure of 0.1 MP and at 121 °C.

Seed was inoculated to the solid-state fermentation medium with 20% inoculum size on the ultra-clean workbench and cultured at 20 °C in the dark. The fermentation product was dried to constant weight at 65 °C, weighed and submitted to superfine grinding.

Component detection method

Determination of crude fiber content

A sample of 1 g was weighed accurately and 200 ml sulfuric acid was added. The mixture was heated to boil for 30 ± 1 min. The concentration of sulfuric acid was 0.255 ± 0.005 mol/L. Then the mixture was filtered, washed with boiling distilled water to neutral pH. 10 mL of petroleum ether was added to the mixture. The procedure was repeated three times. After adding 200 mL of sodium hydroxide to the mixture, it was heated to boil for 30 ± 1 min. The concentration of sodium hydroxide was 0.313±0.005 mol/L. Then the mixture was filtered, washed with boiling distilled water to a neutral pH value. The mixture was put into a crucible, dried to constant weight at 104 °C, and weighed. The crucible was placed in a muffle furnace, burnt at 550 ± 25 °C to constant weight, then weighed and the value was recorded.

$$\text{Crude fiber content} = \frac{a-b}{c} \times 100\% \quad (1)$$

where: *a*: crucible and sample weight before it was burnt (g); *b*: crucible and sample weight after it was burnt (g); *c*: sample weight (g)

$$\text{Crude fiber degradation rate} = \frac{M_1 \times m_1 - M_2 \times m_2}{M_1 \times m_1} \times 100\% \quad (2)$$

where: M_1: weight before fermentation (g); M_2: weight after fermentation (g); m_1: crude fiber content of raw material (%); m_2: crude fiber content of fermented product (%)

The cellulose content of the material (dried to constant weight) and of the fermentation products was determined by GBT6434-2006 "Method for determination of crude fiber feed".

Determination of mannitol content

Nash reagent: 150 g ammonium acetate was weighed accurately and dissolved in distilled water. Then 2 mL of glacial acetic acid and 2 mL of acetyl acetone were added to set the volume to 1000 mL.

0.015 mol/L sodium periodate solution: 3.2 g of sodium periodate was weighed accurately and dissolved in 0.12 mol/L of concentrated hydrochloric acid. The volume was set to 1000 mL.

0.1% L-rhamnose solution: 0.1 g of L-rhamnose was weighed accurately and dissolved in distilled water. The volume of the L-rhamnose solution was set to 100 mL.

100 mg/L mannitol standard solution: 0.1 g of mannitol standard was weighed accurately and dissolved in distilled water. The volume was set to 100 mL with a concentration of 1 g/L. 10 mL mannitol solution with a concentration of 1 g/L was diluted to 100 mg/L.

Preparation of the sample solution: 0.5 g sample dried to a constant weight was accurately weighed and dissolved in 10 mL of distilled water. Then the solution was extracted in a boiling water bath for 2 h and filtered. The residue was washed with distilled water three times. The filtrate solution and washing solution were combined in a volumetric flask. The volume of the solution was set to 100 mL. The solution was named "sample solution".

Method for determination: 100 mg/L standard solution of 0.00 mL, 0.20 mL, 0.40 mL, 0.60 mL, 0.80 mL, 1.00 mL and 0.50 mL of sample solution was used and then made up to 1.00 mL with distilled water. Then 1 mL of 0.015 mol/L sodium periodate was added and mixed at room temperature for 10 min. The solution was then added to 2 mL of 0.1% L-rhamnose solution and well mixed. 4 mL of Nash reagent was added to the solution. Then, the mixture was put in 53 °C water and kept for 15 min. After cooling, the absorbance was measured at the wavelength of 412 nm with a spectrophotometer.

Determination of soluble sugar content

Soluble sugar extraction process: 0.5 g of sample was weighed accurately and 10 mL of distilled water was added at 80 °C with magnetic stirring in a water bath for 2 h. After centrifuging for 10 min with 6000 r/min, the supernatant was collected and the process was repeated twice. The supernatant was combined to 100 mL as the sample solution.

The soluble sugar content of the sample solution was measured by the phenol-sulfuric acid method.

RESULTS AND DISCUSSION

Strain screening

The results of strain screening were presented in Table 1, showing that the crude fiber degradation rates after fermentation were in the following order: ZZYC, DCXC, ZZY, ZZYYS, ZZYZB. The crude fiber degradation rates of ZZYC, DCXC and ZZY reached 33.71%, 28.54% and 18.23%, respectively. The other two revealed a lower yield. However, the mannitol content and the soluble sugar content of

the fermentation product by DCXC were the highest.

In summary, from the analysis of the crude fiber degradation rate, the mannitol content and the soluble sugar content of the fermentation products fermented by different strains, we determined the strain to be used in the experiment to follow – it was *Paecilomyces bainier* (DCXC). It presented great advantages regarding the three indicators. Although the ZZYC had an advantage in the crude fiber degradation rate, it had obviously lower mannitol content and soluble sugar content. Therefore, *Paecilomyces bainier* (DCXC) was preferred as fermentation strain.

Optimization of fermentation conditions

Single factor test: fermentation time

The crude fiber degradation rate varied with an increase in fermentation time, as shown in Table 2. There was a significant change from 0 to 25 days. From 25 to 35 days, it did not change significantly and began to decline after 35 days. The soluble sugar content of the fermentation products had a downward trend in the process from 0 to 10 days, then an upward trend was remarked, while after 35 days it slightly decreased. From 20 to 50 days, the soluble sugar content did not change significantly. The mannitol content first increased and then decreased. At the 25 day duration, it reached the maximum.

In summary, after the analysis of the crude fiber degradation rate, the mannitol content and the soluble sugar content of the fermentation products, considering the utilization of cellulose, the content of the active ingredient of the fermentation product and industrial production costs, the optimum fermentation time was determined as 25 days. If the fermentation time lasted too long, it would increase the cost of industrial production.

Single factor test: inoculum size

As shown in Table 3, the crude fiber degradation rate reached the maximum when the inoculum size was 40% (27.97%). It was 25.38% when the inoculum size was 20%, less by 2.59% than for an inoculum size of 40%. However, the mannitol content and the soluble sugar content reached the maximum when the inoculum size was 20%. Considering the bio-availability of the fermentation products and the costs at an industrial level, the optimum inoculum size was determined as 20%.

Table 1
Experimental results of strain screening

Strains	Weight after fermentation (g)	Crude fiber content (%)	Crude fiber degradation rate (%)	Mannitol content (%)	Soluble sugar content (%)
ZZYC	6.2850±0.0200	45.08±0.28	34.07±0.42	0.72±0.05	4.86±0.65
DCXC	7.6333±0.0101	40.01±1.10	28.54±1.96	2.00±0. 07	8.10±3.44
ZZY	7.8605±0.0439	44.46±0.41	18.23±0.76	1.07±0.03	7.02±1.50
ZZYZB	8.4454±0.0150	46.27±0.29	8.57±0.57	0.51±0.07	7.02±1.08
ZZYYS	8.3281±0.0085	50.28±0.17	2.03±0.33	0.49±0.04	7.09±2.11

Table 2
Experimental results for different fermentation time

Fermentation time (days)	Weight after fermentation (g)	Crude fiber content (%)	Crude fiber degradation rate (%)	Mannitol content (%)	Soluble sugar content (%)
0	10.0000±0.0000	42.74±0.35	0.00±0.00	1.70±0.05	21.45±1.80
5	9.1989±0.0094	46.34±0.46	0.26±0.26	2.17±0.04	13.66±0.65
10	8.1932±0.0259	49.02±0.51	6.03±0.09	2.50±0.05	6.85±0.33
15	7.7430±0.0085	47.34±0.31	14.24±0.08	2.57±0.04	7.24±0.34
20	7.7563±0.0093	46.05±0.29	16.43±0.08	2.74±0.07	9.19±0.54
25	7.5951±0.0081	43.41±0.84	22.86±0.04	2.74±0.10	9.28±0.56
30	7.5101±0.0113	44.25±0.67	22.25±0.06	2.68±0.05	10.03±0.26
35	7.3415±0.0114	44.43±0.64	23.68±0.04	2.27±0.07	10.38±1.83
40	7.4151±0.0046	44.86±0.49	22.17±0.06	1.72±0.05	10.12±0.95
45	7.3745±0.0028	45.40±0.62	21.67±0.06	1.92±0.11	10.60±0.28
50	7.4564±0.0456	45.56±0.84	20.52±0.33	1.83±0.06	10.35±0.51

Table 3
Experimental results for different inoculum size

Inoculum size (%)	Weight after fermentation (g)	Crude fiber content (%)	Crude fiber degradation rate (%)	Mannitol content (%)	Soluble sugar content (%)
10	7.3175±0.0244	44.13±0.33	24.45±0.57	1.76±0.02	9.67±0.66
20	7.4201±0.0163	42.98±0.11	25.38±0.21	1.83±0.02	11.54±0.58
30	7.2297±0.0664	42.90±0.20	27.43±0.34	1.76±0.03	9.71±0.29
40	7.3369±0.0104	41.96±0.30	27.97±0.51	1.77±0.01	9.00±0.30
50	7.4311±0.0116	43.62±0.05	24.16±0.08	1.75±0.01	9.03±0.19
60	7.4028±0.0150	43.19±0.22	25.19±0.38	1.74±0.02	8.45±0.14

Table 4
Experimental results for different fermentation medium pH value

Fermentation medium pH value	Weight after fermentation (g)	Crude fiber content (%)	Crude fiber degradation rate (%)	Mannitol content (%)	Soluble sugar content (%)
2.5	7.2923±0.1065	44.69±0.45	23.91±0.76	1.67±0.08	8.43±0.15
4.0	7.1106±0.0985	42.75±0.16	29.03±0.26	1.70±0.11	8.79±0.03
Initial value	6.9587±0.0733	42.59±0.16	30.80±0.26	1.91±0.07	9.37±0.04
5.5	7.1376±0.0654	43.08±0.49	28.21±0.82	1.81±0.07	8.99±0.12
7.0	7.0332±0.0521	42.73±0.28	29.83±0.45	1.62±0.12	9.69±0.03
8.5	7.2635±0.6231	43.11±0.44	26.89±0.75	1.61±0.13	9.76±0.21
10.0	7.1068±0.3564	49.02±0.91	18.61±0.51	1.70±0.11	9.69±0.13

Single factor test: fermentation medium pH value

As shown in Table 4, the crude fiber degradation rate reached the maximum (30.80%) when the fermentation medium pH value was the initial one (4.8). When the pH value was 7.7, the crude fiber degradation rate was 29.83%, then it increased to 30.80%. We also found that the pH value of the fermentation medium had little effect on the soluble sugar content. With the variation in pH, the mannitol content first increased and then decreased. It reached the maximum of 1.91% at the initial pH value.

From the results obtained, the optimal pH value of the fermentation medium was determined to be the initial pH value, also taking into account the problem of cumbersome production.

Table 5
Experimental results for different fermentation substrate thickness

Fermentation substrate thickness (cm)	Weight after fermentation (g)	Crude fiber content (%)	Crude fiber degradation rate (%)	Mannitol content (%)	Soluble sugar content (%)
0.5	3.9642±0.4654	47.70±0.40	11.52±0.74	2.54±0.05	9.05±0.56
1.0	7.3406±0.3654	42.78±0.32	26.53±0.54	1.81±0.03	9.43±0.46
1.5	11.1095±0.5663	42.88±0.36	25.69±0.60	1.57±0.05	9.16±0.46
2.0	14.7050±0.2663	43.71±0.39	24.81±0.67	1.50±0.04	8.69±0.29
2.5	18.5209±0.5617	43.68±0.25	24.29±0.44	1.47±0.04	8.55±0.44
3.0	21.8820±0.6321	43.55±0.13	25.68±0.22	1.32±0.07	8.04±0.21

Single factor test: fermentation substrate thickness

Table 5 shows the crude fiber degradation rate was the minimum (11.52%) when the fermentation substrate was 0.5 cm thick and the maximum (26.53%) when it was 1.0 cm thick. The difference between the maximum and minimum was of 15.01%. There was little influence of fermentation substrate thickness ranging between 1.0 cm and 3.0 cm on the crude fiber degradation rate. The mannitol content was reduced with an increase in the fermentation substrate thickness. The soluble sugar content first increased and then also reduced with an increase in the fermentation substrate

thickness. It reached the maximum (9.43%) when the fermentation substrate thickness was 1.0 cm, higher than for 0.5 cm thickness. Taking into account the crude fiber degradation rate and the soluble sugar content, we determined the optimum fermentation substrate thickness to be of 1.0 cm.

Pilot scale cultivation of solid fermentation

Using solid-state fermentation technology and new platter fermentation, we cultured 200 boxes, used 40 kg of *Astragalu*s residue and produced 24 kg of fermentation product. The utilization was of 70%.

CONCLUSION

In this study, we used *Cordyceps* as fermentation strain and *Astragalus* residue as fermentation substrate for solid-state fermentation, in order to carry out secondary development of *Astragalus* residue. From the results of the strain screening experiment and fermentation conditions optimization, it was concluded that *Paecilomyces bainier* (DCXC) is the most advantageous as fermentation strain and the optimum fermentation conditions are as follows: fermentation time of 25 days, fermentation substrate pH of the initial value, inoculum of 20% and fermentation substrate thickness of 1.0 cm. New platter fermentation in the pilot scale cultivation has achieved a good effect. The traditional platter fermentation had a lot of disadvantages, such as difficult water retention and oxygen control, pollution and higher culture medium requirement. New platter fermentation avoids these disadvantages.

This work proved that *Astragalus* residue can be used effectively, not only to make use of its active ingredient, but also take advantage of the crude fiber. The success of the pilot-scale cultivation of the *Astragalus* residue solid-state fermentation has laid a good foundation for its industrial production. Further experiments on animals are necessary to take advantage of the fermentation product. To observe animal growth and development, and determine whether it can be used as feed additive, we also provided a new way for the development of new feed additives.

ACKNOWLEDGMENTS: This work was financially supported by the National Spark Key Program of China (2015GA610001), the Foundation of Tianjin University of Science and Technology (Nos. 20120106), the International Science and Technology Cooperation Program of China (2013DFA31160), and the Foundation of Tianjin Educational Committee (20090604).

REFERENCES

[1] Y. Y. Fang and D. L. Fei, *Anim. Breed. Feed*, **70**, 3 (2008).
[2] J. Hua, Z. J. Yang and Z. Y. Zhang, *J. Beijing Agric. Colg*., **55**, 6 (1991).
[3] S. P. Li and X. J. Zhao, *Ecol. Domest. Anim*., **21**, 26 (2005).
[4] R. Zhang, J. H. Zhao, Y. P. Wang, G. Q. Wei and S. Q. Xu, *J. Trad. Chin. Vet. Med*., **48**, 3 (2011).
[5] Y. Z. Jiao, Z. Q. Liang and A. Y. Liu, *Guizhou. Agric. Sci*., **53**, 2 (1990).
[6] Y. Jin, J. Li and M. Z. Fan, *Acta Univ. Med. Anhui*, **20**, 35 (2000).
[7] F. C. Guo, *Mod. Med. Health*, **999**, 22 (2006).
[8] J. Z. Wei, Z. L. Chu and A. H. Chen, *Anim. Sci. Abr*., **5**, 32 (2005).
[9] J. Z. Wei, W. Zhang, Y. Li, M. Z. Fan and G. J. Wang, *Chin. Anim. Husbandry Vet. Med*., **33**, 36 (2009).
[10] A. H. Chen, H. L. Wu, J. G. Zhang, J. Z. Wei and M. Z. Fan, *Food Ferment. Ind*., **129**, 33 (2007).
[11] Z. Y. Liu, *J. Shenyang Agric. Univ*., **105**, 37 (2006).
[12] C. L. Meng, S. F. Wang and F. Zhao, *J. Shenyang Agric. Univ*., **363**, 40 (2009).
[13] C. Z. Dai, X. L. Meng, T. Jiang and M. J. Cheng, *Feed and Animal Husbandry*, **14**, 11 (2011).
[14] L. P. Ooijkaas, F. J. Weber, R. Buitelaar, J. Tamper and A. Rinzema, *Trend. Biotechnol*., **356**, 18 (2000).
[15] T. Robinson, G. McMullan, R. Marchant and P. Nigam, *Bioresour. Technol*., **247**, 77 (2001).
[16] A. Pandey, C. R. Soccol, P. Nigam, V. T. Soccol, L. P. S. Vandenberghe *et al*., *Bioresour. Technol*., **81**, 74 (2000).
[17] A. Pandey, *Biochem. Eng. J*., **81**, 13 (2003).
[18] A. Pandey, C. R. Soccol and D. Mitchell, *Process Biochem*., **1153**, 35 (2000).
[19] A. Durand, R. Renaud, S. Almanza, J. Maratray, M. Diez *et al*., *Biotechnol. Adv*., **591**, 11 (1993).
[20] T. C. Santos, D. P. P. Gomes, R. C. F. Bonomo and M. Franco, *Food Chem*., **1299**, 133 (2012).
[21] G. Panagiotou, D. Kekos, B. J. Macris and P. Christakopoulos, *Ind. Crop. Prod*., **37**, 18 (2003).
[22] P. Velmurugan, H. Hur, V. Balachandar, S. K. Kannan and K. J. Lee, *J. Biosci. Bioeng*., **590**, 112 (2011).
[23] D. Deswal, Y. P. Khasa and R. C. Kuhad, *Bioresour. Technol*., **6065**, 102 (2011).
[24] C. Hsieh and F. C. Yang, *Bioresour. Technol*., **105**, 91 (2004).
[25] S. Q. Yang, Q. J. Yan and Z. Q. Jiang, *Bioresour. Technol*., **1794**, 97 (2006).
[26] C. M. Ajila, S. K. Brar, M. Verma, R. D. Tyagi and J. R. Valéro, *Food Chem*., **1071**, 126 (2011).