Biotech Adv Vol. 11, pp. 495–505, 1993
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0734-9750/93 $24.00
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PROTEIN ENRICHMENT OF SWEET POTATO RESIDUE WITH CO-CULTURE OF AMYLOLYTIC FUNGI BY SOLID-STATE FERMENTATION

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Protein enrichment of sweet potato residue with amylolytic moulds by solid-state fermentation was higher than that obtained with amylolytic yeasts. The optimum initial moisture content for protein enrichment was 66% to 75%. Incrementally added nitrogen sources to the culture at zero time and at 24 h considerably improved the final protein content. During the cultivation, the moisture, ash and ATP contents increased, while the pH value decreased. A 1:1 co-culture of amylolytic mycelial fungi yielded a product with 32.4% crude protein after 4 days incubation at 30°C.

Key words: Protein enrichment, co-culture, amylolytic fungi, ATP content, solid-state fermentation.

INTRODUCTION

The protein demands for animal feeds and also for direct human consumption will inevitably increase during the next decade [11]. In 1991 Taiwan imported 1.96×10^6 tons soybean from the U. S. A. at a cost of 4.93×10^8 U.S. Dollars[4]. Consequently, the developments of local protein resources from renewable raw materials for animal feed is urgently required. The substrates traditionally used in solid state fermentations for the production of foodstuffs are rice, wheat, millet, barley, corn and soybeans. The use of agricultural wastes such as sweet potato residue, which intrinsically is not a good protein source for animal feed, might also be practicable because of its abundant supply, high

productivity and reasonable cost in Taiwan. Furthermore, sweet potato starch is readily converted to biomass by many microorganisms capable of rapid growth[15,17,19].

In order to be economically competitive, it must be possible to perform the bioconversion of starchy materials into protein at rural level. Solid state fermentation facilitates this by reducing the cost of microbial cultivation, by improving the *in vitro* rumen digestibility of substrate, and by increasing the protein and fat contents of starchy or cellulosic materials[5,15,18]. In the previous paper[15], we found that the amylolytic yeasts could enrich the crude protein content of sweet potato residue from an initial value of 6% up to 21% within 3 days by solid state fermentation. In this paper, the protein enrichment of sweet potato residue by solid state fermentation with co-culture of amylolytic fungi was studied.

MATERIALS AND METHODS

Sweet Potato Residue

Sweet potato residue was purchased from the local market in Taiwan, and screened with a 4 to 16 mesh to remove dust and large aggregates. It contained 14.0 to 16.1% moisture, 2.3 to 3.1% crude protein, 2.7 to 3.6% ash, 16.1 to 18.0% crude fibre, and 65.4 to 70.0% carbohydrate[15].

Test Organisms

Saccharomyces cerevisiae Y-187 and Y-191 were provided by C. F. Lin (Microbial Resources Institute, Taiwan); *S. diastaticus* IFO 1046 and *Saccharomyces* sp. IFO 1426 were provided by W. H. Wang (Research Institute for Wines, Taiwan); *Schwanniomyces occidentalis* B53 was provided by J. C. du Preez (Department of Microbiology and Biochemistry, UORS, South Africa); *Candida lipolytica* (*Yarrowia lipolytica*), *Aspergillus niger* Tainan and *Rhizopus* sp. NRRL-688, NRRL-695 and TBR were obtained from our Department. All had amylolytic activities.

Culture Media And Culture Conditions

Amylolytic yeasts were cultivated at 30°C on a slant of modified yeast malt extract medium with soluble starch as carbon source[15], while moulds were cultivated at 30°C on a slant of potato dextrose agar. The solid substrate comprised(g) sweet

potato residue, 100; $(NH_4)_2SO_4$, 1.25; urea, 1.25; and KH_2PO_4, 1.0. The medium was mixed thoroughly with spores or cells and distilled water and incubated statically at 30°C for 4 days by mixing once a day in flask or a home-made column reactor[19]. The pH of the substrate was measured directly by immersing the electrode into the substrate, or determined after mixing an aliquot with 5 vol distilled water.

Measurement Of Adenosine Nucleotide

After cultivation, mash was boiled with 0.02M pH 7.6 Tris buffer for 10 min. The filtrate was used for adenosine nucleotide measurement. Sample solution or adenosine nucleotide solution was mixed with luciferin-luciferase mixture, and the adenosine nucleotide was measured by ATP Photometer (Turner TD-20e Luminomer, USA). ADP and AMP were converted to ATP with pyruvate kinase and adenylate kinase, respectively[8]. Adenosine nucleotide was calculated from the standard curve of authenic compound.

Total Nitrogen, Soluble Nitrogen, Protein, Ash And Moisture Contents

Each sample was extracted with 5 vol distilled water by shaking for 20 min, and soluble nitrogen in the extract was determined directly by the Kjeldahl method[6]. The total nitrogen content of each sample, prior to extraction, was also determined. The crude protein content was calculated from the difference between the total nitrogen and soluble nitrogen contents, using the conversion factor of 6.25[16]. The ash content of samples was determined gravimetrically after 16 to 20 h at 550 to 600°C[13]. The moisture content of the culture was determined by drying a sample at 60°C under vacuum for 8 to 12 h to constant mass[15].

RESULTS

Selection Of Test Organism

Protein enrichment of sweet potato residue with *Rhizopus* sp. NRRL-688, NRRL-695, TBR and *A. niger* Tainan ranged from 14.9 to 18.8%; that with *Schw. occidentalis* B53 and *C. lipolytica* was from 9.6 to 10.3%; and that with *S. cerevisiae* Y-187 and Y-191, *S. diastaticus* IFO 1046 and *Saccharomyces* sp. IFO 1426 was from 5.0 to 7.5%. In all experiments the moisture content of the substrate increased, whereas the pH values decreased. In the light

of these results, *Saccharomyces* sp. IFO 1426, *Schw. occidentalis* B53, *C. lipolytica*, *A. niger* Tainan, and *Rhizopus* sp. NRRL-695 were selected for further study (Table 1).

Initial Moisture Content

Protein enrichment was affected by the initial moisture content which always increased during the cultivation. When *Saccharomyces* sp. IFO 1426 was inoculated into the solid medium with initial moisture content ranging from 55.9 to 79.6%, the final moisture content increased by 0.5% up to 2.9% after 5 days. The protein content gradually increased with an increase in the initial moisture content but reached a maximum value at an initial moisture content of 66.8% and at final moisture content of

Table 1. The protein and moisture contents and pH of sweet potato residue after the growth of different fungi in static solid state culture[1,2]

Test strain	Moisture content (%)		pH		Protein content (%)	
	Initial	Final	Initial	Final	Initial	Final
Saccharomyces sp. IFO 1426	70.4	72.4	5.1	3.7	3.1	7.5
S. diastaticus IFO 1046	69.7	72.8	5.5	3.6	3.2	7.3
S. cerevisiae Y-187	69.0	72.0	4.8	4.4	3.2	5.0
S. cerevisiae Y-191	72.8	74.0	4.6	3.7	3.2	5.6
Schw. occidentalis B53	69.0	76.7	5.2	3.0	3.4	10.3
C. lipolytica	68.1	78.2	5.7	3.2	3.5	9.6
A. niger Tainan	72.6	83.0	4.8	4.5	3.3	18.8
Rhizopus sp. NRRL-688	69.8	78.4	4.6	4.3	3.1	16.9
Rhizopus sp. NRRL-695	69.4	75.5	4.5	4.1	3.4	17.0
Rhizopus sp. TBR	70.6	74.9	4.5	4.0	3.2	14.9

1. All the data are the mean of experiments in triplicate.
2. Each gram of dry substrate inoculated with 10^7 to 10^8 spores or cells, supplemented with 1.25% $(NH_4)_2SO_4$ and 1.25% urea as combined nitrogen sources and incubated at 30^oC for 4 days.

Table 2. The effect of the initial moisture content on the protein enrichment of sweet potato residue in flask culture at 30°C for 5 days

Test strain	Moisture content (%)		pH		Protein content (%)	
	Initial	Final	Initial	Final	Initial	Final
Saccharomyces	55.9	56.4	4.8	4.3	3.2	4.0
sp. IFO 1426	59.2	60.9	4.6	3.8	3.2	5.6
	66.8	69.7	4.5	3.8	3.2	8.4
	71.3	73.1	4.9	3.8	3.2	7.6
	75.2	77.6	4.9	4.0	3.2	7.2
	79.6	82.2	4.7	4.0	3.2	7.1
Rhizopus sp.	55.5	58.4	5.1	4.8	3.8	17.8
NRRL-695	59.6	63.6	5.2	4.4	3.6	17.8
	65.3	68.6	5.1	4.2	3.8	18.0
	68.8	74.2	5.1	4.5	3.8	18.5
	75.6	78.2	5.1	4.6	3.3	18.6
	80.3	83.7	5.1	4.6	3.4	18.6

1. All the data are the mean of experiments in triplicate.
2. The inoculum size was 10^7 to 10^8 spores or cells per gram of dry substrate.

69.7%. In all cases the substrate pH decreased markedly. While *Rhizopus* sp. NRRL-695 was used with initial moisture content ranging from 55.5 to 80.3%, the protein content also gradually increased with an increase in the initial moisture and reached a plateau at an initial moisture content of 68.8%. The substrate pH decreased during the cultivation. The optimum initial moisture content appeared to be in the region of 65 to 75% (Table 2).

Nitrogen Supplementation

To improve the efficiency of utilization of the nitrogen source, a mixture of ammonium sulphate and urea (at a 1:1 ratio) was added to the substrate with 1.25% dry weight of each at zero time and again at 24 h instead of just 2.5% of each nitrogen source addition at zero time. The addition of the nitrogen sources in two increments greatly enhanced the degree of protein enrichment resulting in a final protein content of 17.0 to 29.4%, while the nitrogen sources had only been added at the start of the cultiva-

tion having in a final protein content of 8.6 to 18.2%. The highest protein contents (29.4% and 26.1%) were obtained with the moulds *Rhizopus* sp. NRRL-695 and *A. niger* Tainan, whereas the maximum protein contents obtained with *Schw. occidentalis* B53, *C. lipolytica*, and *Saccharomyces* sp. IFO 1426 was only 18.2%, 17.2%, and 17.0%, respectively.

Column Reactor

Protein enrichment in a column reactor with *Saccharomyces* sp. IFO 1426 and *A. niger* Tainan is shown in Table 3. The moisture content increased by 0.8 to 3.6%, and by 8.1 to 9.7% over 4 days with *Saccharomyces* sp. IFO 1426 and *A. niger* Tainan, respectively. The pH values decreased from the initial values of 4.3 to 4.4 to final values in the range of pH 3.1 to 3.8. The *Saccharomyces* strain gave a final protein content of 7.9 to 8.9%, whereas the value obtained with *A. niger* was 18.1 to 18.8%. Protein enrichment with nitrogen supplementation after 24 h incubation in column reactor clearly showed that the additional supply of nitrogen greatly enhanced microbial growth and protein content. The *Saccharomyces* strain gave a final protein content of 17.5% with nitrogen supplementation, whereas the value obtained with *Schw. occidentalis* B53 was 17.8% (Table 4). These results were also very consistent with the protein enrichment in the flask

Table 3. The change in moisture and protein contents and pH in a column reactor with different initial moisture content at 30°C for 4 days[1]

Test strain	Moisture content (%)		pH		Protein content (%)	
	Initial	Final	Initial	Final	Initial	Final
Saccharomyces sp.	64.3	67.9	4.3	3.8	3.2	8.6
IFO 1426	70.5	72.9	4.3	3.8	3.2	7.9
	72.4	74.4	4.4	3.7	3.2	8.5
	75.0	75.8	4.3	3.2	3.2	8.9
A. niger Tainan	65.4	74.3	4.3	3.5	3.3	18.1
	70.2	80.0	4.4	3.1	2.9	18.7
	72.3	82.0	4.4	3.2	3.0	18.8
	75.0	83.1	4.4	3.2	2.8	18.6

1. Culture conditions were same as Table 1 described.

Table 4. Protein enrichment of sweet potato residue with mono- and co-culturesof different fungi in a solid state cultivation at 30°C with nitrogen supplementationat 24 h [1]

Test organism	Culture period (day)	Moisture content (%)	pH	Ash (%)	AMP (nM/g)	ADP (nM/g)	ATP (nM/g)	Energycharge (ATP+1/2ADP) / (AMP+ADP+ATP)	Protein content (%)
(A) FLASK CULTURE									
Saccharomyces sp.	0	64.1	5.5	4.6	5.7	2.4	1.0	0.24	3.6
IFO 1426	2	64.2	6.0	4.8	27.1	16.1	11.7	0.36	12.0
	4	67.8	5.7	5.7	38.6	31.6	23.8	0.42	17.0
Schwanniomyces	0	64.2	4.7	-	-	-	0.1	-	3.3
occidentalis B53	2	68.6	3.6	-	-	-	40.3	-	12.6
	4	68.4	3.7	-	-	-	26.7	-	18.2
Candida lipolytica	0	64.3	4.4	-	-	-	0.1	-	4.1
	2	65.1	6.8	-	-	-	2.3	-	11.5
	4	67.0	7.4	-	-	-	8.3	-	17.2
Aspergillus niger Tainan	0	63.4	5.4	4.2	5.0	2.7	1.1	0.28	3.7
	2	73.8	3.3	6.9	94.2	32.4	24.4	0.27	17.1
	4	77.1	6.4	8.8	353.1	179.3	35.7	0.22	29.4
Rhizopus sp. NRRL-695	0	64.4	5.5	4.6	5.3	2.9	0.8	0.25	3.4
	2	72.5	4.8	6.8	596.4	278.5	45.7	0.20	16.6
	4	75.5	8.3	7.2	317.2	260.7	70.2	0.31	26.1
(B) COLUMN REACTOR									
Saccharomyces sp.	0	63.3	5.1	4.3	-	-	0.3	-	4.3
IFO 1426	2	65.2	4.9	4.8	-	-	6.4	-	11.7
	4	68.8	5.0	5.5	-	-	17.6	-	17.5
Schwanniomyces	0	63.8	4.8	4.6	-	-	0.4	-	4.6
occidentalis B53	2	68.5	4.5	5.5	-	-	29.0	-	12.1
	4	71.3	4.4	6.0	-	-	27.9	-	17.8
Saccharomyces sp. IFO	0	64.5	5.5	4.4	5.4	2.8	1.1	0.27	3.6
1426 and C.lipolytica	2	65.5	5.9	4.9	62.2	32.7	14.8	0.28	12.8
	4	65.6	7.6	4.6	182.9	158.9	36.8	0.31	18.8
Schw. occidentalis B53	0	63.8	4.5	-	-	-	0.1	-	4.1
and C. lipolytica	2	69.2	5.1	-	-	-	22.5	-	12.5
	4	71.3	6.3	-	-	-	15.7	-	18.6
Saccharomyces sp. IFO	0	64.5	5.5	4.6	5.2	2.9	0.9	0.26	3.5
1426 and Rhizopus sp.	2	69.8	4.3	5.9	236.2	246.0	21.0	0.29	16.2
NRRL-695	4	72.7	4.2	6.4	308.6	214.2	23.2	0.24	25.9
Schw. occidentalis B53	0	64.1	4.7	-	-	-	0.1	-	4.1
and Rhizopus sp. NRRL-	2	73.4	4.6	-	-	-	28.3	-	14.4
695	4	76.8	8.5	-	-	-	19.5	-	26.2
C. lipolytica and	0	64.9	5.3	4.8	5.3	3.2	1.0	0.27	3.6
Rhizopus sp. NRRL-695	2	70.9	4.5	6.4	735.4	236.1	36.8	0.15	12.7
	4	76.1	8.1	8.2	301.7	213.7	40.1	0.27	26.7
A. niger Tainan and	0	64.1	5.4	4.5	5.5	2.6	0.7	0.23	3.6
Rhizopus sp. NRRL-695	2	73.5	4.6	6.9	731.6	256.8	47.6	0.17	15.7
	4	78.5	6.8	8.3	395.2	241.4	54.0	0.25	32.4
Blank	0	64.2	4.6	4.6	5.0	2.4	0.7	0.23	3.4
	2	64.5	4.4	4.7	4.4	2.1	0.6	0.23	3.3
	4	64.0	4.7	4.6	4.2	2.2	0.4	0.23	3.4

1. The culture conditions were as in Table 3, but with supplementation with $(NH_4)_2SO_4$ 1.25% and urea 1.25% at 24 h.

culture.

Co-culture of Amylolytic Fungi

Protein enrichment of sweet potato residue with mono-culture or with a 1:1 co-culture of amylolytic fungi (by the number of spores or cells) at 30°C for 4 days is shown in Table 4. Moisture content of the final products with the mono-culture of amylolytic moulds was higher than that with amylolytic yeasts; while in the case of co-culture of amylolytic moulds and amylolytic yeasts, moisture content of the final products was the same as that of mono-culture of amylolytic moulds. During the incubation, the substrate pH of co-culture of amylolytic fungi also decreased initially then increased later. The mono-culture of *Rhizopus* sp. NRRL-695 and *A. niger* Tainan had the products with 26.1 to 29.4% of protein for 4 days cultivation, while *C. lipolytica*, *Saccharomyces* sp. IFO 1426 and *Schw. occidentalis* B53 had the products with 17.0 to 18.2% of protein. Co-culture of these two amylolytic fungi had the products with 32.4% of protein. Protein enrichment of co-culture of amylolytic yeasts was the same as that of mono-culture and the same phenomena was found in the co-culture of amylolytic moulds. In the case of the co-culture of amylolytic yeasts and amylolytic moulds, protein content in the final product was intermediate between the values of the mono-cultures.

ATP And Ash Contents In The Solid Medium

To investigate the biomass content in the solid substrate, the ATP content was measured and is shown in Table 4. During the cultivation, ATP content increased. *Rhizopus* sp. NRRL-695 had the highest ATP content in the medium, then the co-culture of amylolytic moulds and the mono-culture of *A. niger* Tainan. While *C. lipolytica* was the least. The energy charge also increased with time. *Saccharomyces* sp. IFO 1426 had the highest value, while *A. niger* Tainan had the least.

Ash content increased during incubation, *A. niger* Tainan had the highest value, then the co-culture of *A. niger* Tainan and *Rhizopus* sp. NRRL-695, and *Saccharomyces* sp. IFO 1426 had the lowest. The final protein content of products of the mono-culture of amylolytic moulds, and the co-culture of amylolytic mould and amylolytic yeast was between 25.9 and 29.4%, and the final protein content of the mono-culture of amylolytic yeasts and the co-culture of amylolytic yeasts was between 17.0 and 18.8%.

DISCUSSION

In 1991 Taiwan cultivated 12,819 ha of sweet potato with the annual production 224,272 tons. The productivity was 1.75 ton/ha [4]. Sweet potato residue contains 2.3% protein and 65.4% total carbohydrate and is, in itself, not a good resource of protein for animal feeding. However, it could be enriched with protein by using amylolytic fungi by a solid state fermentation process[11, 15]. In a previous paper, we found that amylolytic yeasts enriched the protein of sweet potato residue from the initial protein 6% to the final protein 21% for 2 to 3 days incubation[15]. In this paper, it was found that amylolytic moulds had a higher potential than the amylolytic yeasts.

During the fermentation, the moisture content of substrate increased. The increase might be due to the production of metabolic water of fungi, or the release of water in oxidation of carbohydrate, as had been observed in the spawn of mushroom[14], protein enrichment of sugar beet pulp[3, 18], and antibiotics production of sweet potato residue[19]. Microbial utilization of the ammonium sulfate as the nitrogen source can cause the pH decrease[2]. When nitrate or urea serves as nitrogen sources, the pH value rose due to the reduction of nitrate to $R\text{-}NH_3^+$ or urea decomposition[9]. During the fermentation, the pH initially decreased, but subsequently increased. pH control in solid substrate may be obtained by using different ratios of ammonium salts and urea [15], ammonium nitrate or sodium nitrate[17], or a buffering agent such as $CaCO_3$[10].

In order to enrich the protein content from 3-6% to 20-30%, an addition of 4% nitrogen supplementation is necessary theoretically. In this study, we found that the fractional supplementation of nitrogen could result in a higher protein enrichment than supplementation added only at zero time.

ATP is a potential index of microbial biomass[7], and energy charge is a useful indicator of the energetic state of cells[12]. ATP content increased during the cultivation. ADP and AMP contents also increased as incubation proceeded except for the mono-culture of *Rhizopus* and the co-culture of amylolytic fungi and *Rhizopus*. Energy charge of the mono-culture or the co-culture of amylolytic fungi was between 0.15 and 0.42. The value of energy charge was lower than that of yeast cells growing anaerobically before early stationary phase or yeast cells growing aerobically during log phase and stationary phase[1]. The low value of energy charge in solid state fermentation might be due to the low concentration of oxygen or glucose in the

substrate. The oxygen concentration in the solid substrate was only 5% and 3% at 24 h and 48 h incubation, respectively. Ball and Atkinson also indicated that the energy charge was 0.14 to 0.40 in anaerobic condition and long-term starvation[1]. Since the microbes and substrate were static in solid state fermentation, therefore the nutrient supply might be also a limiting factor for low value of energy charge.

The use of the filamentous fungi proved to be much better at enriching the protein content of the sweet potato residue than the yeasts. This was probably mainly because the moulds, through growth of their hyphae, were better able to penetrate and spread through the solid substrate. The use of co-cultures of the moulds, yeasts, or a mould with a yeast failed to enhance the protein enrichment. A column reactor gave results comparable to those obtained in static flask cultures.

ACKNOWLEDGEMENT

The author thanks Professor James C. du Preez of University of the Orange Free State, Bloemfontein, South Africa for his helpful comments, and the National Science Council of the Republic of China for financial support (NSC 79-0409-B002-52 and NSC 80-0409-B002-27).

REFERENCES

1. W. J. Ball Jr. and D. E. Atkinson, Adenylate energy charge in *Saccharomyces cerevisiae* during starvation. J. Bacteriol., 121. 975-982.

2. C. L. Cooney, Growth of microorganisms, in Biotechnology, H. J. Rehm and G. Reed (Eds), Verlag Chemie, Weinheim, Vol. 1, 73-112 (1981).

3. A. Durand and D. Chereau, A new pilot reactor for solid-state fermentation: application to the protein enrichment of sugar beet pulp, Biotechnol. Bioeng., 31. 476-486 (1988).

4. Economics and Planning Department, Custom Statistics-1991 Taiwan, Taipei, Taiwan, R.O.C. (1992).

5. G. A. Grant, Y. M. Han and A. W. Anderson, Pilot-scale semi-solid fermentation of straw, Appl. Environ. Microbiol., 35. 549-553 (1978).

6. W. Horwitz, Cereal food, in Official Methods of Analysis of the Association of Official Analytical Chemists, 13th ed. p. 211 (1980).

7. D. M. Karl, D. R. Jones, J. A. Novitsky, C. D. Winn and P.

Bossard, Specific growth rates of natural microbial communities measured by adenine nucleotide pool turnover, J. Microbiol. Methods, 6. 221-235 (1987).

8. A. Lundin and A. Thore, Comparison of methods for extraction of bacterial adenine nucleotides determined by firefly assay, Appl. Microbiol., 5. 713-721 (1975).

9. D. A. Mitchell, H. W. Doelle and P. F. Greenfield, Agar plate growth studies of *Rhizopus oligosporus* and *Aspergillus oryzae* to determine their suitability for solid state fermentation, Appl. Microbiol. Biotechnol., 28. 598-602 (1988).

10. I. D. Reid, Solid state fermentations for biological delignification, Enzyme Microbiol. Technol., 11. 786-803 (1989).

11. J. C. Senez, M. Raimbault and F. Deschamps, Protein enrichment of starchy substrate for animal feeds by solid state fermentation, World Anim. Rev., 35. 36-39 (1980).

12. E. Sivori, Adenylic nucleotides and energy charge during the embryonic development of *Bufo arenarum*, Comp. Biochem. Physiol., 85B. 573-576 (1986).

13. The AVI Publishing Company Inc, Food Analysis Laboratory Mannual, West Port, Connecticut, 85-86, and 102 (1975).

14. H. H. Wang, Water absorption characteristics of cellulosic wastes and solid state fermentation, Proc. 2nd World Congr. Chem. Eng., 297-300 (1981).

15. S. S. Yang, Protein enrichment of sweet potato residue with amylolytic yeasts by solid state fermentation, Biotechnol. Bioeng., 32. 886-890 (1988).

16. S. S. Yang, S. L. Chang, C. B. Wei and H. C. Lin, Reduction of waste production in the Kjeldahl method, J. Biomass Energy Soc. China, 10. 147-155 (1991).

17. S. S. Yang and W. F. Chiu, Protease production with sweet potato residue by solid state fermentation, Chin. J. Microbiol. Immunol., 19. 276-288 (1986).

18. S. S. Yang, A. Durand and H. Blachere, Protein enrichment of sugar beet residue with conidia of *Trichoderma album* by solid state fermentation, Chin. J. Microbiol. Immunol., 19. 11-22 (1986).

19. S. S. Yang and S. S. Yuan, Oxytetracycline production by *Streptomyces rimosus* in solid state fermentation of sweet potato residue, World J. Microbiol. Biotechnol., 6. 236-244 (1990).