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Protein Enrichment of Potato Starch Residue by Solid State Fermentation with Mixed Strains

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Abstract: This study was to select strains which have a strong ability to degrade starch and strain which has a strong ability to synthesize protein from nine strains of three species for mixed strains fermentation of potato starch residue to enrich the protein content for further use as an animal feed and to investigate the optimal combination of selected strains and technical parameters of fermentation process. In the experiment of single strain fermentation, three strains, *Candida tropicalis* AS 13, *Saccharomycopsis fibuligera* JS2 and *Geotrichum candidum* AS 2.2342 were selected for mixed strains fermentation by comparison the degradation rate of starch and the synthesis rate of protein. In the experiment of mixed strains fermentation, the True Protein (TP) content were obtained in the substrate fermented by the three strain combination was higher than two-strain combinations. Then the effects of technical parameters such as inoculation ratio, inoculation method and total inoculum size of solid state fermentation on the TP content were analyzed, respectively. The experiment results showed that the use of potato starch residue fermented with the three-strains combination of *C. tropicalis* AS 13, *S. fibuligera* JS2 and *G. candidum* AS 2.2342 at the ratio of 1:7:2 with total 10 mL inoculum size at the same time can improve TP content by up to 16.1% which would subsequently provide a good feedstuff for ruminants.

Key words: Potato starch residue, solid state fermentation, mixed strains fermentation, true protein, feed stuff

INTRODUCTION

With the rapid development of animal husbandry, the demand for feed, especially protein feed has increased each year and has become an urgent problem that cannot be neglected. In the recent years, much interest has been focused on biotechnological potential of converting agro-industrial wastes for protein feed such as detoxifying the cottonseed meal by solid substrate fermentation with *C. tropicalis* ZD-3 and *A. niger* ZD-8 (Zhang *et al*., 2006), protein enrichment of potato pulp (Gelinas and Barrette, 2007), apple pomace (Vendruscolo *et al*., 2009) and cassava pulp (Kaewwongsa *et al*., 2011; Thongkratok *et al*., 2010).

In China, >2 million ton of Potato Starch Residue (PSR) are produced during the production of starch from potatoes (Liu *et al*., 2010; Ren and Zhang, 2011). PSR contains starch, cellulose, hemicelluloses, pectin, protein and other minor constituent. With the exception of using it as animal feed in a dried form traditionally, PSR is also used for extracting pectin, dietary fiber and soluble fiber, etc., by physical or chemical means but it is difficult to achieve industrialization because these methods are either high cost and low efficiency or generate waste liquid and secondary environment pollution. It is necessary to explore the utilization of PSR for valuable products by costeffective method. This stimulates interest in bioconverting PSR into commercially value-added products. Wang *et al*. (2010) used potato pulp as substrates for the cultivation of *Geotrichum candidum*, lactic acid bacteria and yeast for protein-enriched poultry feed by solid state fermentation.

Solid State Fermentation (SSF) is defined as the fermentation involving solids in absence (or near absence) of free water (Pandey, 2003). Compared with Submerged Fermentation (SmF), although SSF has some disadvantages such as heat and mass transfer is not homogeneous (Mitchell *et al*., 2000), it might have many advantages over SmF, such as superior productivity, low waste water output and improved product recovery (Uyar and Baysal, 2004). Due to the absence of free water, the desired products by SSF do not need further purification which is necessary after SmF because water needs to be separated from the products by energy-consuming techniques such as centrifugation, evaporation or filtration (Vendruscolo *et al*., 2008). SSF processes are usually used for the production of organic acid (Hofrichter *et al*., 1999), enzyme (Sharma and Arora, 2010) and single cell protein (Anupama and Ravindra, 2001), etc.

The main objective of this study was to select strain which has a strong ability to degrade starch and strain which has a strong ability to synthesize protein from nine strains of three species and to investigate the optimal

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combination of selected strains and technical parameters of fermentation process for solid state fermentation of PSR with mixed strains.

MATERIALS AND METHODS

Potato starch residue: PSR which was obtained from Youfound Foodstuff Co., Ltd, Shanxi Province, China contained 19.2% dry matter and 38.3% raw starch, 7.4% soluble suger, 2.2% ash, 4.7% ether extract, 16.2% crude fiber, 9.1% crude protein and 6.8% True Protein (TP) on dry matter basis.

Microorganisms: Nine strains used in this study were obtained from the Chinese Academy of Sciences for feed fermentation including six strains of two yeast species (*Candida tropicalis* AS 7, *Candida tropicalis* AS 13, *Candida tropicalis* BK 49, *Saccharomycopsis fibuligera* XZ 1-5, *Saccharomycopsis fibuligera* XZ 5-4, *Saccharomycopsis fibuligera* JS2) and three strains of one fungi species (*Geotrichum candidum* AS 2.2339, *Geotrichum candidum* AS 2.2342 and *Geotrichum candidum* AS 2.2356). All microorganisms are stored at 4°C in the refrigerator with Potato Dextrose Agar (PDA) slant medium.

Culture media and condition: PDA slant medium was used for activating the strains at 28°C in the incubator for 2 days.

YPD liquid seed medium: About 1 g yeast extract, 2 g peptone, 2 g glucose dissolved in 100 mL distilled water in 250 mL triangular flasks was used for cultivating the liquid seed using a rotary shaker at 28°C at 120 rpm for 2 days.

PSR was used as the basic substract in solid state fermentation. About 32 g PSR was moistened with mineral water which contain 1 g urea, 0.2 g K_2HPO_4, 0.1 g $MgSO_4.7H_2O$, 0.05 g $MnSO_4.H_2O$ and 0.01 g $Fe_2(SO_4)_3$ in a 500 mL triangular flask, the initial moisture content and pH were adjusted with phosphate buffer to about 65 and 7.0%, respectively.

All the above medium were sterilized at 0.1 Mpa for 15 min, then cooled to room temperature before inoculated.

SSF of PSR: All strains were inoculated from seed medium to the solid state medium, thoroughly mixed with a sterile glass rob and sealed with sealing membrane. They were then cultivated statically at 28°C and meanwhile, the humidity was controlled at about 65% in a constant temperature and humidity incubator. At the end of fermentation, the product was dried at 60°C for 48 h.

Chemical and statistical analysis: In the mixed SSF medium, only part of the inorganic nitrogen source was transformed to TP, so the remaining inorganic nitrogen source would interfere with protein determination and to some extent will make the measured value higher. To solve this problem, TP was precipitated by trichloroacetic acid precipitate method (Licitra *et al*., 1996) and then measured by Kjeltec Method (AOAC, 1995). Starch was measured by enzymatic-colorimetric procedure (Rasmussen and Henry, 1990).

Statistical analysis: Results obtained from the experiment were subjected to one-way analysis of variance using the General Linear Model procedure of the SAS (2004) Software Package, the significance of difference was tested by LSD test and was declared at $p<0.05$.

RESULTS AND DISCUSSION

SSF of PSR by single strain: The starch and TP contents of the fermented PSR by single strain were shown in Table 1. The starch content of the substrate was decreased whereas TP content was increased after fermented by all microorganisms. Starch in the substrate was utilized sharply by three strains of *S. fibuligera* and most by *S. fibuligera* JS2. So, *S. fibuligera* JS2 was selected for mixed strains fermentation. The content of TP was increased more obviously by three strains of *G. candidum* than the strains of *C. tropicalis* and *S. fibuligera* and highest by *G. candidum* AS 2.2342, significantly more than the results of all other strains included *G. candidum* AS 2.2356 and *G. candidum* AS 2.2356 ($p<0.05$). Therefore, the strain *G. candidum* AS 2.2342 was chosen for further study of mixed strains fermentation. In addition, although the ability of starch degradation of *C. tropicalis* was weaker than *S. fibuligera* and the ability of protein synthesis was poorer than *G. candidum*, the decreasing rate of the starch content of the substrate was significantly greater than any one strain of *G. candidum* ($p<0.05$) and the increasing range of the

Table 1: Starch and True Protein (TP) content of potato starch residue fermented by single strain

		C. tropicalis			*S. fibuligera*			*G. candidum*		
Microorganism	Control	AS 7	AS 13	BK49	XZ1-5	XZ5-4	JS2	AS2.2339	AS2.2342	AS2.2356
Starch (%)	35.5^{a}	28.3^{d}	25.4^{e}	29.1^{cd}	20.0^{g}	21.1^{f}	15.6^{h}	29.6^{c}	28.4^{d}	31.1^{b}
TP (%)	6.4^{g}	7.7^{de}	8.2^{c}	7.4^{f}	7.9^{d}	7.6^{e}	7.9^{d}	8.7^{b}	9.2^{a}	8.8^{b}

$^{a-g}$Means in the same row with different superscripts differ ($p<0.05$)

TP was significantly greater than all strains of *S. fibuligera* ($p<0.05$). Considering both the ability of starch degradation and protein synthesis, the strain *C. tropicalis* AS 13 was also retained for further study of mixed strains fermentation of PSR.

SSF of PSR by mixed strains; Different combination in process of SSF: Table 2 showed the TP content of the fermented PSR by mixed strains with different combinations, the TP content increased greatly when the substrate were fermented by mixed strains. As supported by the rapid growth of mixed cultures, the highest TP content obtained in the substrate fermented by the three-strain fermentation, the increase rate was as high as 106.1%, higher ($p<0.05$) than two-strain and single strain fermentation (Table 1 and 2).

The inoculation ratio of seed culture in process of SSF: PSR was fermented for 48 h by inoculated total 5 mL liquid seed of *C. tropicalis* AS 13, *S. fibuligera* JS2 and *G. candidum* AS 2.2342 according to the ratio of the Table 3. It was observed that the highest ($p<0.05$) TP content was 14.3% when the inoculation ratio of *C. tropicalis* AS 13: *S. fibuligera* JS2: *G. candidum* AS 2.2342 was 1:7:2, the increase rate of TP reached the maximum. And the lowest ($p<0.05$) TP content was obtained when the inoculation ratio was 1:8:1.

Inoculation method in process of SSF: Table 4 showed that when *C. tropicalis* AS 13, *S. fibuligera* JS2 and *G. candidum* AS 2.2342 were inoculated at the same time, the TP content was significantly more than other inoculation method ($p<0.05$). On the contrary, the lowest ($p<0.05$) TP content was obtained when *G. candidum* AS 2.2342 was inoculated on the substrate which was inoculated *C. tropicalis* AS 13 and *S. fibuligera* JS2, 24 h and with sterilization. The inoculation methods were as follows:

Table 2: True Protein (TP) content of potato starch residue fermented by mixed strains

Combinations of strains	TP (%)
Control	6.4^{e}
C. tropicalis AS 13 + *G. candidum* AS 2.2342	11.4^{c}
S. fibuligera JS2 + *G. candidum* AS 2.2342	12.9^{b}
C. tropicalis AS 13 + *S. fibuligera* JS2	9.4^{d}
C. tropicalis AS 13 + *S. fibuligera* JS2 + *G. candidum* AS 2.2342	13.2^{a}

The inoculum size of two strain combination was 2.5+2.5 mL of three strain combination was 1.25+1.25 + 2.5 mL. $^{a-e}$Means in the same row with different superscripts differ ($p<0.05$)

- *C. tropicalis* AS 13, *S. fibuligera* JS2 and *G. candidum* AS 2.2342 were inoculated at the same time
- *G. candidum* AS 2.2342 was inoculated on the substrate which was inoculated *C. tropicalis* AS 13 and *S. fibuligera* JS2 12 h and then with sterilization
- *G. candidum* AS 2.2342 was inoculated on the substrate which was inoculated *C. tropicalis* AS 13 and *S. fibuligera* JS2 12 h and without sterilization
- *G. candidum* AS 2.2342 was inoculated on the substrate which was inoculated *C. tropicalis* AS 13 and *S. fibuligera* JS2 24 h and then with sterilization
- *G. candidum* AS 2.2342 was inoculated on the substrate which was inoculated *C. tropicalis* AS 13 and *S. fibuligera* JS2 24 h and without sterilization

Inoculum size in process of SSF: The effect of different inoculum size on TP content was studied as shown in Table 5. TP content was 9.4% when inoculum size was 1 mL and gradually increased with the increasing of inoculum size and up to maximum point ($p<0.05$) until the inoculum size was 10 mL then decreased to 14.3 and 12.8% when inoculum size was 12 and 15 mL, respectively.

Experiment of single strain fermentation implied that *S. fibuligera*, especially *S. fibuligera* JS2 has a strong ability to produce amylase and degrade starch, this result was consistent with previous study in which high amylase activity of crude enzyme preparations from *S. fibuligera* grown with gelatinised cassava starch was determined by viscometric assay (Gonzalez *et al*., 2002). *G. candidum* was often used for studying the production of single cell protein as it has a strong ability to synthesize protein by fermenting the cheap agro-industrial wastes (Curto *et al*., 1992; Ziino *et al*., 1999), this study found that *G. candidum* AS 2.2342 was the best strain for synthesizing cell protein by SSF of PSR. *C. tropicalis* AS 13 was selected for further study of mixed strains fermentation of PSR due to both the relatively strong ability of starch degradation and protein synthesis.

Experiments of mixed strains fermentation implied that the higher yield of TP was probably due to the enzymatic hydrolysis of the starch and the protein synthesis by the combination of *C. tropicalis* AS 13, *S. fibuligera* JS2 and *G. candidum* AS 2.2342. On the one hand, reducing sugar which was released by *C. tropicalis* AS 13 and *S. fibuligera* JS2 could be efficiently metabolized by

Table 3: The effect of inoculation ratio on True Protein (TP) content of potato starch residue fermented by mixed strains

Ratio of three strains	Control	1:8:1	1:7:2	2:6:2	1:6:3	2:5:3	3:4:3	1:5:4	2:4:4	1:4:5	2:3:5
TP (%)	6.4^{i}	11.2^{h}	14.3^{a}	13.6^{c}	14.1^{b}	13.9^{bc}	13.1^{d}	12.9^{de}	12.7^{ef}	12.5^{f}	12.0^{g}

The order of the ratio of three strains was *C. tropicalis* AS 13: *S. fibuligera* JS2: *G. candidum* AS 2.2342. $^{a-i}$Means in the same row with different superscripts differ ($p<0.05$)

Table 4: True Protein (TP) content of potato starch residue fermented by mixed strains with different inoculation method

Inoculation method	Control	A	B	C	D	E
TP content(%)	6.4^{f}	14.3^{a}	12.5^{c}	13.4^{b}	11.0^{e}	12.3^{d}

$^{a-f}$Means in the same row with different superscripts differ ($p<0.05$)

Table 5: The effect of inoculum size on True Protein (TP) content of potato starch residue fermented by mixed strains

Inoculation size (mL)	0	1	2	5	8	10	12	15
TP (%)	6.4^{g}	9.4^{f}	11.4^{e}	14.1^{c}	15.0^{b}	16.1^{a}	14.3^{c}	12.8^{d}

$^{a-g}$Means in the same row with different superscripts differ ($p<0.05$)

G. candidum AS 2.2342 which raised TP content in the substrate with sufficient carbon source on the other hand, the utilization of reducing sugar can reduce the feedback inhibition effect of reducing sugar on the amylase accelerating the degradation of starch. This result agreed with the standpoint of Bader *et al.* (2010) that the utilization of cocultures appears to be advantageous over a single microorganism because of the potential for synergistic utilization of the metabolic pathways of all involved strains in a coculture situation. Coculture fermentation instead of single cultivation has been widely used to produce many products (Mapari *et al.*, 2005; Shrestha *et al.*, 2009), it resulted in not only increased yields but also a reduction of process costs due to inexpensive substrates (Kleerebezem and Loosdrecht, 2007). Aziz and Mohsen (2002) used sweet potato residue as a substrate to produce microbial protein by submerged fermentation relusts showed that when sweet potato residue fermented by mixed culture of *Fusarium moniliforme* and *Saccharomyces cerevisiae* for 3 days, the free sugar was utilized efficiently and rapidly and the maximal biomass yield (13.96 g L^{-1}) and protein production (65.8%) were obtained.

In the case of the inoculation ratio of *C. tropicalis* AS 13: *S. fibuligera* JS2: *G. candidum* AS 2.2342 was 1:7:2, the reducing sugar from amylase hydrolyzation was utilized by *G. candidum* AS 2.2342 immediately and thereby the feedback inhibition was relieved and the fermentation process proceeded with the metabolism of starch degradation and protein synthesis. But by only raising the inoculation ratio of amylase-produced yeast (*C. tropicalis* AS 13 and *S. fibuligera* JS2) or protein-synthesized fungi (*G. candidum* AS 2.2342), the reducing sugar production from starch and protein synthesis from reducing sugar could not match appropriately, therefore the TP content was at the lower level. Bhalla and Joshi (1994) investgated protein enrichment of apple pomace by co-culture of cellulolytic moulds and yeasts and found that the highest yield of protein was obtained when substrate was seeded with 2.5 mL cell suspension of *Candida utilis* and 2.5 mL *Aspergillus niger*. Similar reason was found that the enzymatic hydrolysis of the lignocellulosic component of the pomace by the *A. niger* released free sugar which *C. utilis* could efficiently metabolise.

When *C. tropicalis* AS 13, *S. fibuligera* JS2 and *G. candidum* AS 2.2342 were inoculated at the same time, *G. candidum* AS 2.2342 could utilized the reducing sugar in the initial substrate at the beginning of fermentation and provided the necessary growth factor for *C. tropicalis* AS 13 and *S. fibuligera* JS2 and in the growth process of *C. tropicalis* AS 13 and *S. fibuligera* JS2 reducing sugar was produced by the amylase hydrolyzation for *G. candidum* AS 2.2342 to synthesize cell protein. Subsequently, symbiosis was well established and the whole ability of the protein-producing system was improved. In the contrary, in the report of Xiao *et al.* (2009) who studied *Vallisneria natans* for crude protein by SSF with *Aspergillus niger* and *Candida utilis* with different inoculation method, the results showed that the lowest protein production was obtained when *A. niger* and *C. utilis* was inoculated at the same time but the highest protein production was obtained when *C. utilis* was inoculated later than *A. niger*. This might be due to the substrate and microorganisms were different.

Inoculum size as an important factor was studied by many researchers (Danesh *et al.*, 2011; Li *et al.*, 2009). The first increased and then decreased trend was observed by Irfan *et al.* (2011) who obtained the maximum crude protein production from wheat bran by *Candida utilis* with 10% inoculum size. When the inoculum size was not excessive, the TP content would increase with the increasing of inoculum size whereas excess of inoculum size would lead to increased viscosity of the substrate and ineffetive mass transfer and lower yield.

CONCLUSION

Mixed strains fermentation increased the TP content of the PSR substrate and the highest protein content (16.1%) was obtained from the substrate of mixed fermentation by *C. tropicalis* AS 13, *S. fibuligera* JS2 and *G. candidum* AS 2.2342 at the ratio of 1:7:2 with total 10 mL inoculum size at the same time. PSR has the potential to be used as solid substrate for protein enrichment by *C. tropicalis* AS 13, *S. fibuligera* JS2 and *G. candidum* AS 2.2342.

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