Austin Journal of Biotechnology & Bioengineering

Open Access

Austin Publishing Group

Rapid Communication

Solid-State Fermentation of Soybean and Rice Processing Coproducts with *Thermothelomyces thermophila* for Protein Enrichment

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Received: November 12, 2018; **Accepted:** December 28, 2018; **Published:** January 04, 2019

Abstract

The purpose of this research is to develop a protein enrichment technology that can be used for feed production. Soybean meal, rice husk and bran were used as a substrate for solid-state fermentation with *Thermothelomyces thermophila* fungi to evaluate the protein increase during fermentation process. The proportion of substrate components was balanced to get the best result. The effects of moisture content and additional nitrogen sources on the value of protein gained was investigated. The average protein grows was about 3%.

Keywords: Soybean meal; Rice bran; Solid-state; Fermentation; Protein; Enrichment

Introduction

Animal breeding as the part of agricultural industry is major economic sector that provide food supply security of the state. Population growth push up demand for food and makes agricultural sector to drive up food production. As a result, we can see competitiveness between feedstock and food-stock agricultural sectors. Fertile lands are limited and soon it will not be enough to cover all the needs for feeding and food cultures. The other problem is a demand for quality and cheap feed to satisfy modern pedigree cattle's needs. Low cost source of protein required to produce animal feeds.

Microorganisms such as bacteria, algae and fungi are potential substitute of plant protein for feeds production as they consist of 30-70% protein of valuable amino acid content. Bacteria are widely used in biotechnology for amino acid production, yeast are used to produce feed protein, but fungi appliance in feed production is still poor. Fungi are mostly used as hemicellulose or other ferments producers, but high content of protein make it possible to use fungi as source of feed protein [1]. Besides, specific medium composition is needed for yeast and bacteria to cultivate, when agricultural wastes that can be used as a fungi cultivating medium make cultivating process comparatively cheap.

Far Eastern Federal District has large number of cultivated fields, used to grow rice and soybean. Soybean processing wastes (soybean meal) are used for feed production, as it consist of 40-50% of protein. Rice processing wastes (bran and husk) have low nutrition and usually utilized as wastes [2].

Researchers in many countries held similar experiments to evaluate the effect of fungi fermentation on nutrition level of substrate: Iowa state university experiment with soybean coproducts fermentation with A. oryzae, T. reesei and P. chrysosporium showed an absolute increase of protein content of 1, 4-3, 2% [3]. The researchers from Danish Technological Institute got an absolute increase of protein content by 0, 8-4% using cassava residue as a substrate for *Trichoderma pseudokoningii* [4]. During Ibadan university experiment, the protein content was increased from 4% to 10% with Trichoderma viride and cassava residue based substrate [5]. Similar researches under the project for developing solutions for feed protein producing technology are held in Russia at G.B. Elyakov Pacific Institute of Bioorganic Chemistry (PIBOC) and Far Eastern Federal University (FEFU) [1].

This research is aimed at improving the protein content of agricultural wastes and coproducts available in the territory of Primorsky region using *Thermothelomyces thermophila*.

The work has been done with financial support from Ministry of Education and Science of Russia (Agreement 02.G25.31.0172 dated 01.12.2015).

Materials and Methods

Soybean meal, rice bran and husk in variety of combinations were used as substrate for fungi. Rice bran and husk were sampled from rice processing plant in Spasskii district. Soybean meal (GOST R 53799-2010) was bought in the market of Ussuriysk.

Dry matter content of the samples was measured using standard method weighting before and after drying for 8 hours at 110°C in an oven.

Raw protein content was detected by measuring the total nitrogen content using Kjeldahl method and calculated by multiplying the

Table 1: Solid-state fermentation substrate composition % of value.

No.	Rice bran	Soybean meal	Rice husk
1	20%	70%	10%
2	30%	60%	10%
3	40%	50%	10%
4	50%	40%	10%
5	60%	30%	10%
6	70%	20%	10%

Citation: Podvolotskaya AB, Rochin EO and Tekuteva LA. Solid-State Fermentation of Soybean and Rice Processing Coproducts with *Thermothelomyces thermophila* for Protein Enrichment. Austin J Biotechnol Bioeng. 2019; 6(1): 1102.

Table 2: Absolute protein increase value by substrate content and moisture content.

Sub./No.	True protein % before fermentation	True protein after fermentation			
		60 ml of liquid		120 ml of liquid	
		% of true protein	True protein absolute increase	% of true protein	True protein absolute increase
1	36,4%	37,5%	1,1%	37,9%	1,5%
2	32,6%	34%	1,4%	34,2%	1,6%
3	29,2%	30,9%	1,7%	30,8%	1,6%
4	25,1%	27,3%	2,2%	27,4%	2,3%
5	22,5%	24,8%	2,3%	25,1%	2,6%
6	19,3%	21,4%	2,1%	21,7	2,4%

Table 3: The influence of additional nitrogen sources on protein growth in the substrate.

	Control sample	No adding sample	Adding sample:	
			CH_4N_2O	$NaNO_3$
Protein in dry matter	22,5%	25,1%	24,9%	25,4%
Protein increase	-	2,6%	2,4%	2,9%

result with 6.25 index. Non-protein nitrogen content was measured using Bernstein method. True protein content was calculated by subtracting non-protein content from raw protein content.

Amino acid content was determined using capillary electrophoresis system "Kapel'-105M".

Mycotoxins content was measured using Bio Scientific ELISA test kits for determination of aflatoxin B1, DON, Zearalenone, T-2 toxins.

Thermothelomyces thermophila was obtained from G.B. Elyakov Pacific Institute of Bioorganic Chemistry (Vladivostok, Russia). The culture was grown on petri plates with Czapek Dox agar media (Himedia) at 44°C for 5 days. Then the culture from petri plates was inoculated in slant plain agar tubes (Himedia). The tubes were kept at 30°C and reinoculated every 7 days.

To prepare the inoculum the culture from the tubes was transferred and grown at petri plates with Czapek Dox agar media for 5 days at 44°C. Then small round pieces of Czapek Dox agar (Ø=8mm) cut from petri plates and containing the culture was inoculated in 200 ml glass bottles with 120ml of Czapek Dox media (Himedia) in it. The bottles were stored at 44°C for 5 days and used as inoculum.

Substrate components were mixed in defined proportions (Table 1), put into craft paper bag and sterilized by autoclaving at 121°C for 20 minutes.

Rice husk was added to provide better aeration. Sterile substrate was divided among sterile single 1,2 L containers 140 gm each.

At the first step, containers with substrates were inoculated with 60 ml of inoculum. The half of the containers were added 60 ml of sterile water. The content of the containers were stirred with sterile spreader. The containers have been incubated for 10 days at 40°C. Bottle with sterile water was placed near the containers to prevent drying.

At the second step, the effect of additional nitrogen sources was investigated. The substrate, showed the best protein increase, at the step one was chosen. 0.8 g of $NaNO_3$ or 0.3g of CH_4N_2O were added. The doses were calculated according to the Czapek Dox broth media composition (3 g/l) and single container substrate mass (260 g). The dose of CH_4N_2O were recalculated according to the percentage of nitrogen. Containers were incubated with the same conditions.

Table 4: Amino acid profile of *Thermothelomyces thermophila*.

Amino acid	%
Lysine	2,2±0,7%
Valine	2,1±0,8%
Threonine	1,1±0,4%
Methionine	0,3±0,1%
Leucine + isoleucine	3,2±0,9%
Histidine	2,7±0,8%
Phenylalanine	1,3±0,4%

After the end of the fermentation process, the content of the containers was transferred to craft-paper bags and sterilized at 110°C for 15 minutes.

1L laboratory glass fermenter was used for liquid-state fermentation. 600 ml of sterile Czapek Dox broth media (Himedia) was placed at sterile fermenter. Small pieces of Czapek Dox agar media (Himedia) with *Thermothelomyces thermophila* were added. During multiply of experiments, fermentation process was held with different settings of stirring rate (0-60 rpm), aeration (0-0.2 l/min) and temperature (30-50°C). Liquid-state fermentation was carried from 7 to 21 days.

Results and Discussion

After the first step of the solid-state fermentation, the optimal content of substrate that provided the highest absolute increase of protein was defined (Table 2).

The optimal composition of the substrate in this case is a mixture consisting of 60% of the rice bran, 30% of soybean meal and 10% of rice husk - the largest absolute increase in protein was 2.6%

A larger increase in protein was observed in the samples the sterile water was added. It was concluded that additional moisture promoted a better growth of the fungus.

The mycotoxins content tests showed negative result for the presence of toxins controlled in animal feeds (aflatoxin B1, DON, Zearalenone, T-2 toxins).

The test's results showed that sodium nitrate, added to the substrate as a source of nitrogen, had a weakly positive effect on the protein gain during fermentation; however, this influence can be called insignificant (Table 3).

The addition of urea to the substrate did not have a positive effect on the protein gain.

It was noted that mixing during liquid-stage fermentation had a negative effect on the growth of the mycelium. The fermentation temperature change in the range from 30°C to 44°C does not significantly affect the mycelium growth. The adhesive properties of fungus was noticed during the liquid-stage fermentation, the main growth of the mycelium was observed in the near-wall zone and the boundary with the air. The dry mass of *Thermothelomyces thermophila* from 1 liter of liquid medium was from 0.7 to 1.5 g for 2 weeks. Protein in dry matter of the fungus is 45%. Essential amino acids content was measured (Table 4).

Aminoacid composition of fungi dried mass shows high percentage of lysine, valine, leucine+isoleucine and histidine acids.

Conclusion

The experiments results showed, that under conditions given, the maximal protein increase after the solid-state fermentation with Thermothelomyces Thermophilla using rice husk, soybean meal and rice bran as a substrate averaged about 3%. Calculations are needed to evaluate the economic efficiency of technology represented, but supposedly, 3% increase cannot return on the costs for the process. The researches concerning usage of additional nitrogen by fungi may be perspective, but according to Bekker research the average inorganic nitrogen consumption of 1 g fungi dried equivalent mycelia is about 1 g per hour [6]. According to the results, adding 3 g/l $NaNO_3$ has no significant positive effect on mycelia growth. The level of inorganic nitrogen in substrate should be designated carefully, as higher concentration may provide inhibiting effect on fungi. The others factors can have an effect on fungi mass growth should be investigated. Substrate spatial configuration inside fermenter tank is another point that has to be researched thoroughly.

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