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Detoxification of castor bean residues and the simultaneous production of tannase and phytase by solid-state fermentation using *Paecilomyces variotii*

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ABSTRACT

In this work, we introduce a biological detoxification method that converts toxic waste from castor beans into animal feed material. This method simultaneously induces the production of tannase and phytase by *Paecilomyces variotii*; both enzymes have high levels of activity and have the potential to be used in feedstuffs because they decrease overall anti-nutritional factors. The maximum tannase and phytase activities obtained were 2600 and 260 U/g after 48 and 72 h, respectively. SDS–PAGE electrophoresis of the fermented castor cake extracts revealed a reduction in ricin bands during fermentation, and the bands were no longer visible after 48 h. The cytotoxicity of the extracts was evaluated by MTT testing on RAW cells, and a progressive increase in cellular viability was obtained, reaching almost 100% after 72 h of fermentation.

1. Introduction

The castor bean contains oil that makes up approximately 50% of the mass of the bean (w/w, dry mass). The oil has special characteristics, such as high viscosity, heat and pressure stability, a low freezing point, and the ability to form waxy substances after chemical treatments (Conceição et al., 2005). The castor bean is a promising candidate for biodiesel production; in recent years, Brazilian research has focused on the development of a process to produce biodiesel from castor beans (Gutarra et al., 2005). After a transesterification reaction, an unwanted by-product referred to as castor bean residue is produced (Godoy et al., 2009).

The castor bean residue, or cake, that remains after the extraction of the oil comprises about one half of the castor bean's weight (Robb et al., 1974). The residue has a protein content of 34–36%. When the beans are decorticated, the protein content of the cake can be increased to 60% (Mottola et al., 1968), potentially making it an excellent source of protein for animals. Despite its availability and high protein content, castor bean residue is not used as a protein supplement due to its toxicity; currently, it is used as an organic fertilizer. Ricin is the most lethal of the toxins found in castor bean residue and is reported to make up as much as 1.5% (w/w, in defatted cake). The other three toxins present in castor bean residue are ricinine, *Ricinus* communis agglutinin and allergen CB-1A. These toxins are present in lower concentrations and have relatively insignificant toxic effects, rendering them negligible when considering the use of castor bean residue as animal feed. For this reason, any attempt to detoxify castor bean residue should be aimed at removing ricin (Anandan et al., 2005).

Oil cakes, such as castor bean residue, are potentially very useful for biotechnological enzyme production. There are several reports describing the production of various enzymes using oil cakes as substrates in solid-state fermentation (SSF), or as a supplement to the production medium. Oil cakes are an ideal nutrient support in SSF because they provide both carbon and nitrogen sources, and are reported to be good substrates for enzyme production in fungal species (Ramachandran et al., 2007).

The production of feed-grade enzymes such as amylases, cellulases, tannases and phytases by solid-state fermentation (SSF) and their application in feed have been examined in many studies (Bogar et al., 2002; Gautam et al., 2002; Sabu et al., 2002; Singhania et al., 2009).

Phytases are the key enzymes used to supplement animal feed and have been used as animal growth boosters (fish, poultry, swine) by pre-treating or dephytinizing feedstuffs (Cao et al., 2007). The myo-inositol hexakisphosphate 3-phosphohydrolase (EC 3.1.3.8), and myo-inositol hexakisphosphate 6-phosphohydrolase (EC 3.1.3.26), are classified as histidine acid phosphatases that catalyze the hydrolysis of phosphate from phytic acid, producing inorganic phosphate and myo-inositol phosphate derivatives. The released phosphorus can then be used in metabolic processes. These enzymes are present in many plant and animal tissues, and they are also produced by many species of fungi and bacteria (Singhania et al., 2009).

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Tannin acyl hydrolase, commonly referred to as tannase (EC 3.1.1.20), is an enzyme that cleaves ester linkages in hydrolysable tannins (Belmares et al., 2004), producing glucose and gallic acid (Banerjee et al., 2005). Tannase is an extracellular, inducible enzyme produced in the presence of tannic acid by fungi, bacteria and yeast (Battestin et al., 2007a). This enzyme increases the bioavailability of nutrients by the hydrolysis of the phenolic (tannins) anti-nutritional factors when used during the preparation of feed (Graminha et al., 2008).

In this work, *Paecilomyces variotii*, which has previously reported to be an excellent tannin-degrading microorganism and tannase producer (Battestin and Macedo, 2007b), was used to produce tannase and phytase simultaneously, both of which are very important enzymes used during the production of animal feed. Castor bean residue, a toxic residue and byproduct of Brazilian biodiesel production, was inoculated with a strain of *P. variotii* for enzymatic production using the solid-state fermentation process.

We demonstrate that it is possible to combine the biotechnological production of these two enzymes, which are important in the feed enrichment process, with the biological detoxification of the castor bean residue, enabling its use as a rich protein source in animal feed.

2. Methods

2.1. Microorganism and inoculum preparation

The *P. variotii* strain used in this study was isolated from soil samples collected in the state of São Paulo, Brazil, and was selected as the tannase and phytase producer. The strain was deposited at the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI) under the number 1157.

The fungus strain was maintained in a Potato Dextrose Agar (PDA) medium on slants with glycerol, which were refrigerated at 4 °C. For sporulation, plates with PDA medium were inoculated with the fungal strain and incubated at 30 °C for 3 days. The spores were suspended in distilled water at a concentration of 9×10^6 spores/mL (Battestin et al., 2007a).

2.2. Solid-state fermentation

A. Azevedo Industry Oils (Itupeva, SP) donated the castor bean residue. The residue was ground in a knife mill (Philips, RI 1725) and separated in a sieve shaker (*Mesh* 10, particle size under 1.86 mm). The pH of residue was obtained by homogenization in water using a Digimed DM-22 potentiometer; the free water from the residue was measured using a Karl Fischer Analyzer KF-1000. The fermentation medium was prepared in 250 mL Erlenmeyer flasks, where 5 g of the castor bean residue was added to 5 mL of saline solution containing (g/L): KH_2PO_4, 1.0; NH_4NO_3, 2.0, $MgSO_4{\cdot}7H_2O$, 0.2; $CaCl_2{\cdot}2H_2O$, 0.02; $MnCl_2{\cdot}4H_2O$, 0.004; $Na_2MoO_4{\cdot}2H_2O$, 0.002; $FeSO_4{\cdot}7H_2O$, 0.0025 and 10% tannic acid (w/w). After sterilization, the flasks were inoculated with 1 mL of the inoculum suspension and were incubated at 30 °C and 90% relative humidity for 96 h.

2.3. Enzyme extraction

After incubation, acetate buffer (pH 5.0, 0.02 M) was added, and the flasks were shaken at 200 rpm for 1 h. The solution was filtered and centrifuged at 10,070 *g* for 30 min at 4 °C (Centrifuge Beckman J2-21, Beckman-Coulter, Inc., Fullerton, CA, USA). The supernatant was assayed for phytase and tannase activity. The material retained on the filter was assayed for hydrolysable tannins, and the presence of ricin was evaluated by electrophoresis and a cytotoxicity assay.

2.4. Enzymatic activity assays

Phytase and tannase activities were evaluated according to Stockmann et al. (2003) and Mondal et al. (2001), using *p*-nitrophenylphosphate and tannic acid as substrates, respectively. One unit of enzymatic activity was defined as the amount of enzyme needed to release 1 μmol/min of free *p*-nitrophenol when evaluating phytase, and 1 μmol/min of residual tannic acid when evaluating tannase. Enzyme activity was expressed as units/gram of dry, solid medium (based on initial mass).

2.5. Culture conditions for the production of tannase and phytase

The fermentation parameters that had the greatest influence on tannase production were evaluated with a CCD (central composite design) methodology, and the same analysis was performed to evaluate the influences on phytase production. The independent variables were relative humidity (%) (Climate Chamber 420 CLD – Nova Etica, SP, Brazil), saline solution concentration (% (v/w) of total dry substrate) and tannic acid concentration (% (w/w) of total substrate). The concentration of the saline solution was determined according to the maximum moisture absorption capacity of the castor bean residue. The variables were coded, according to Eq. (1):

$$x_i = \frac{X_i - X_0}{\Delta X_i}. \quad (1)$$

Here, x_i is the coded variable, X_i is the natural variable of the nutrient factor, X_0 is the value of the natural variable at the center point, and ΔX_i is the step change value. The variables and levels are shown in Table 1.

The Statistica software package defined a full CCD methodology for 3 factors (2^3), consisting of 8 cubic points, 6 star points and 3 replicates at the center point, which served to estimate experimental error and to investigate the suitability of the proposed model, the details of which are presented in Table 1. The experimental results were fitted to a second-order polynomial function, and the Student *t*-test permitted checking the statistical significance of the regression coefficients. Analysis of variance (ANOVA) was performed on the experimental data to evaluate the statistical signif-

Table 1
Coded levels (in parentheses) and real values for the experimental design and results of CCD.

Trial	Relative humidity (%)	Saline solution (v/w)(%)	Tannic acid (w/w)(%)	Enzymatic activity (U/g)	
	X_1 (x_1)[a]	X_2 (x_2)	X_3 (x_3)	Tannase (48 h)	Phytase (72 h)
1	−1 (66)	−1 (31)	−1 (6)	1536	70
2	+1 (84)	−1 (31)	−1 (6)	2292	168
3	−1 (66)	+1 (49)	−1 (6)	496	8
4	+1 (84)	+1 (49)	−1 (6)	864	35
5	−1 (66)	−1 (31)	+1 (10)	796	43
6	+1 (84)	−1 (31)	+1 (10)	824	113
7	−1 (66)	+1 (49)	+1 (10)	872	10
8	+1 (84)	+1 (49)	+1 (10)	816	20
9	−1.68 (60)	0 (40)	0 (8)	480	10
10	+1.68 (90)	0 (40)	0 (8)	1720	151
11	0 (75)	−1.68 (25)	0 (8)	1432	117
12	0 (75)	+1.68 (55)	0 (8)	416	22
13	0 (75)	0 (40)	−1.68 (4.6)	1544	28
14	0 (75)	0 (40)	+1.68 (11.4)	688	29
15	0 (75)	0 (40)	0	1304	19
16	0 (75)	0 (40)	0	1208	22
17	0 (75)	0 (40)	0	1152	18

[a] x_i is the coded value and X_i is the actual value of the it independent variable. The conversion between x_i and X_i is described on Eq. (1).

icance of the model. The response model was expressed in terms of coded variables, ignoring the statistically non-significant terms.

2.6. Evaluation of hydrolysable tannin content

The hydrolysable tannin content of castor bean residue, before and after fermentation, was extracted using 50% aqueous acetone. The mixture was shaken at 150 rpm for 2 h. After shaking, the mixture was filtered and centrifuged at 1320*g* for 15 min at 5 °C, and the clear supernatant was analyzed (Naczk and Shahidi, 2004). The hydrolysable tannin content was analyzed according to Brune et al. (1991). The test was done in triplicate.

2.7. Ricin detection by protein electrophoresis – detoxification detection

Castor bean residues were prepared as described in item 2.3 (enzyme extraction). The materials were solubilized (4:1) in a phosphorus buffer (pH 7.0) for 3 h in a rotary shaker and centrifuged at 10,000 *g* for 15 min. The samples were then dissolved in a loading buffer (Tris–HCl with β-mercaptoethanol and sodium dodecyl sulfate) at a concentration of 10 mg/mL, and were boiled for 5 min at 95 °C. The samples were analyzed by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (Vertical Slab Mini-Protean Electrophoresis System Bio-Rad Laboratories, Hercules, CA, USA) according to Laemmli (1970). The proteins were stained with a 0.5% solution of Coomassie brilliant blue R-250. Control references were as follows: (M) standard MW electrophoresis calibration kit (Pharmacia Biotech), (R) pure standard ricin sample, (1) unfermented castor bean residue extract and (2) unfermented and autoclaved castor bean residue extract. Both castor bean residue extract controls were dissolved in a loading buffer at a concentration of 10 mg/mL, and were boiled for 5 min at 95 °C.

2.8. Determination of cytotoxicity

RAW 264.7 cells were used to inoculate 96-well plates at a density of 10×10^4 cells per well. Following a 24-h incubation period, adherent cells were washed once with PBS (phosphate-buffered saline). Cells were then incubated in media containing various concentrations of unfermented castor bean residue extract to define the minimum toxic concentration. Positive and negative cellular controls were also included in this analysis. The cell viability among the cultures was analyzed after a 24-h incubation period using the MTT method (Mosmann, 1983). The plate was centrifuged for 10 min at 1200 rpm at 4 °C. After removing the medium, 10 μL of MTT solution and 90 μL of PBS were added to each well of an ELISA plate, and the plate was incubated at 37 °C for 3 h. Following the incubation, 100 μL of 10% SDS in 0.01 M HCl was dispensed into each well using a pipette to dissolve the formazan, and the plates were incubated for 18 h. Finally, the absorbance of each well was measured at 540 nm using an ELISA plate reader.

After the minimum toxic concentration of the castor bean residue extract had been defined, it was used in the MMT test, which was repeated as described above, but with samples of biotransformed castor bean residue extract taken after 24, 48 and 72 h of fermentation with *P. variotii*.

3. Results and discussion

3.1. Tannase and phytase production in castor bean residue

The wild Brazilian *P. variotii* strain was able to grow in castor bean residue by solid-state fermentation, which resulted in the production of the extracellular enzymes tannase and phytase. The maximum activities occurred after 72 h (phytase) and 48 h (tannase), and these times were used in the CCD experiment.

An experiment was designed to determine the optimal relative humidity (%), saline solution concentration (% (v/w) of total dry substrate) and tannic acid concentration for solid-state fermentation, and the results are shown in Table 1.

The quadratic model used to calculate the tannase activity, after eliminating the statistically insignificant terms ($p > 0.1$), is as follows:

$$Y = 1152 + 233x_1 - 301x_2 - 84x_2^2 - 243x_3 - 144x_1x_3 + 317x_2x_3$$

The analysis of variance was reproduced and is shown in Table 2. The Fisher *F*-statistic ($F = 25.3 > F_{t0.1;6;10} = 2.46$) was 10.3 times higher than the F_t and *p*-value of <0.01, demonstrating that this regression model was statistically significant at the 90% confidence level. Additionally, the R^2 value obtained was 0.94.

The highest tannase activity achieved in the experimental design tests was 2292 U/g at 84% relative humidity, 31% (v/w) saline solution content and 6% (w/w) tannic acid concentration (assay 2). The response surface in Fig. 1 indicates that higher tannase activity may have been found at higher relative humidity levels; however, relative humidity levels higher than 90% were not attainable in the Climate Chamber, limiting the highest experimental value of relative humidity to 90%.

The trends observed for the saline solution concentration and tannic acid concentration variables indicated that higher enzymatic activity was obtained using lower concentrations. Previous studies suggest that greater increments of inducer molecules can have toxic effects on *P. variotii*, among other issues (Battestin and Macedo, 2007b), which may explain why lower concentrations of tannic acid were more effective at inducing tannase production. A tannic acid concentration of 6% (w/w) was significantly lower than the value of 11% used by Battestin and Macedo (2007b), who produced tannase using the same microorganism grown on wheat bran and coffee husk. Using a lower tannic acid concentration reduces the cost of the medium.

Additionally, tannase production decreased as the concentration of saline solution was increased. One hypothesis was that the lower enzymatic activity was due to the presence of salts in the solution, and not to the moisture levels that the solution contained. To confirm this idea, a single variable fermentation test was performed with trials containing a 25% (v/w) saline solution or 25% (v/w) distilled water. A humidity of 25% (v/w) is low enough that it does not inhibit fungal growth. The fixed conditions were 90% relative humidity and 6% (w/w) tannic acid concentration. The results of tannase activity for this test were 2.092 and 2.620 U/g for the media containing salt solution and distilled water, respectively. Inorganic salts can change osmotic balance (Zeng et al., 2011) and can either inhibit or enhance microbial growth. This phenomenon has not been observed in previous studies using wheat or coffee husk to induce tannase production by *P. variotii* (Battestin and Macedo, 2007b) but can be explained by the

Table 2
Analysis of variance and regression analyses[a] for the response of the central composite design of tannase production.

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F_{test}[b]	*p*-Value
Regression	3845263	6	640877	25.31	0.0001
Residual	253209	10	25321		
Lack of fit	241390	8	30174		
Pure error	11819	2	5910		
Total	4098472	16			

[a] Coefficient of determination: $R^2 = 0.94$.
[b] $F_{t0.1;6;10} = 2.46$.

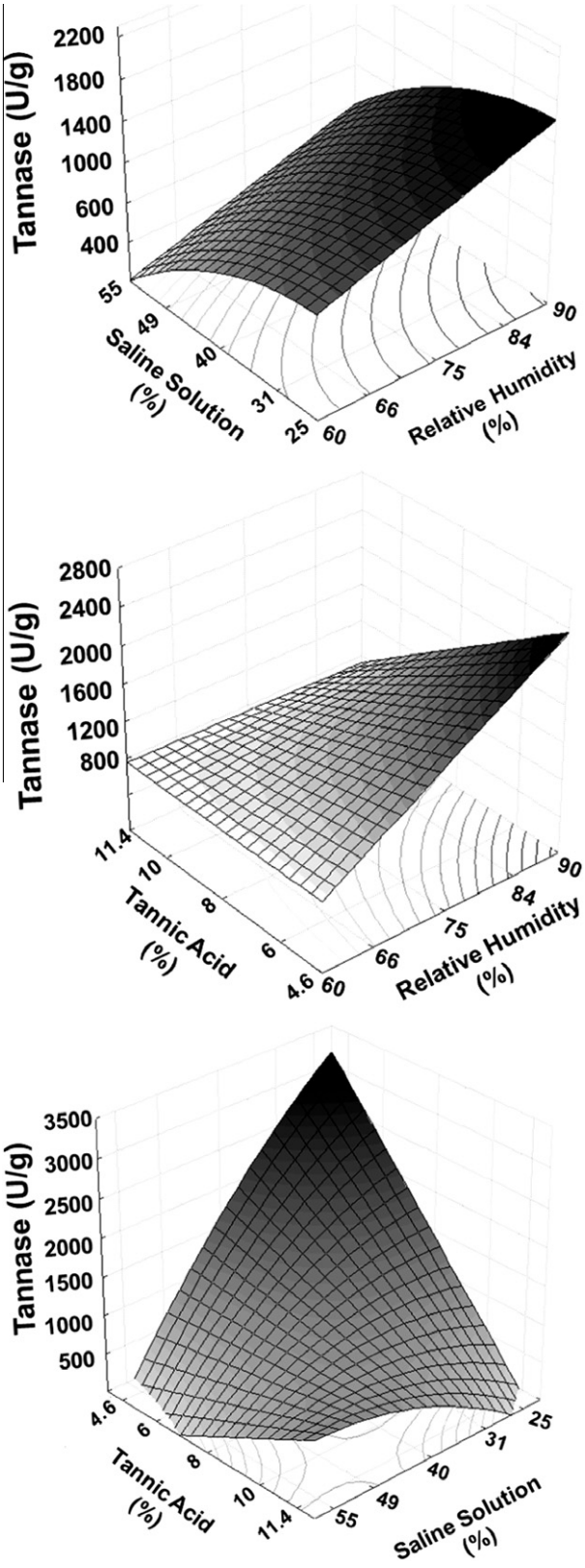

Fig. 1. A response surface representative of tannase activity as a function of relative humidity vs. tannic acid concentration vs. the concentration of saline solution, according to the CCD.

reduced mobility of substrates during the solid-state fermentation process as described by Ito et al. (2011).

The tannase activity achieved in these experiments was significantly higher than the activities obtained by Sabu et al. (2005), who obtained 13 U/g after 96 h of fermentation at 30 °C with the solid-state fermentation of palm kernel cake using *Aspergillus niger*, or than the 69 U/g reported by Kumar et al. (2007), who used *Aspergillus ruber* to produce tannase at 30 °C after 96 h, with Amla, Ber, Jamun, Jamoa and Keekar leaves as substrates. These data demonstrate the high potential for tannase production by this wild strain isolated by our group, data which are reinforced by the findings of Battestin and Macedo (2007b).

Phytase production by *P. variotii* was analyzed after the fermentation process had proceeded for 72 h. The results are shown in Table 1. The quadratic model calculated for maximum phytase activity, after eliminating the statistically insignificant terms ($p > 0.1$), is as follows:

$$Y = 23 + 32x_1 + 20x_1^2 - 35x_2 + 16x_2^2 - 16x_1x_2$$

The analysis of variance reproduced in Table 3 shows that the model was significant. The Fisher F-statistic ($F = 25.5 > F_{t0.1;5;11} = 2.45$) was 10.4 times higher than the F_t value, and the p-value of <0.01 demonstrates that this regression model is statistically significant at the 90% confidence level. The R^2 value obtained for this model was 0.92.

The greatest phytase activity was 184 U/g at 84% relative humidity, 31% (v/w) saline solution and 6% (w/w) tannic acid. Similar to what was observed in the tannase production model, Fig. 2 indicates that higher phytase activities could be obtained with a relative humidity above 90% and by maintaining the saline solution below 25% (v/w). The effect of tannic acid content was ignored because phytase production was not influenced by its presence.

The same steps described for the evaluation of tannase production were followed when evaluating phytase production, and a single variable fermentation test was performed with trials containing a 25% (v/w) saline solution or 25% (v/w) distilled water. The fixed conditions were also 90% relative humidity and 6% (w/w) tannic acid. The results of phytase activity for this test were 240 and 265 U/g for the media containing salt solution and distilled water, respectively. The fermentation conditions most favorable for fungal growth and tannase production were also the best conditions for the phytase production in the same medium, the only difference being that maximum tannase activity was obtained after 48 h and that the maximum phytase activity was obtained after 72 h.

There are a few studies on phytase production by solid-state fermentation in the literature. However, the phytase activity we achieved was higher than that reported by Vassilev et al. (2007), who obtained 58 U/g of activity from dry olive waste inoculated with *A. niger*, and it was also higher than the 44.5 U/g published by Roopesh et al. (2006), who produced phytase using *Mucor racemosus* with solid-state fermentation using sesame oil cake and wheat bran as substrates at 30 °C, after 96 h of fermentation.

The enzymatic activity levels obtained for both tannase and phytase in these experiments demonstrates the potential for this process to be used in industrial applications. In previous studies, we demonstrated that tannase and phytase have synergistic effects that decrease anti-nutritional factors (tannins and phytates) in animal feed (Schons et al., 2011). It is very interesting that we were able to produce both enzymes using the same experimental process.

3.2. Analysis of biotransformed castor bean residue

The analysis of hydrolysable tannins from the castor bean residue extracts before and after fermentation by *P. variotii* showed a reduction from 3.3 ± 0.2 to 0.5 ± 0.2 mg/g of substrate. Tannins are the substrates of the tannase produced by the tested fungus, and their hydrolytic action during the fermentation may have

Table 3
Analysis of variance and regression analyses[a] for the response of the central composite design of Phytase production.

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F_{test}[b]	*p*-Value
Regression	40085	5	8017	25.53	0.00001
Residual	3450	11	314		
Lack of fit	3441	9	382		
Pure error	9	2	4.5		
Total	43535	16			

[a] Coefficient of determination: $R^2 = 0.92$.
[b] $F_{t0.1;5;11} = 2.45$.

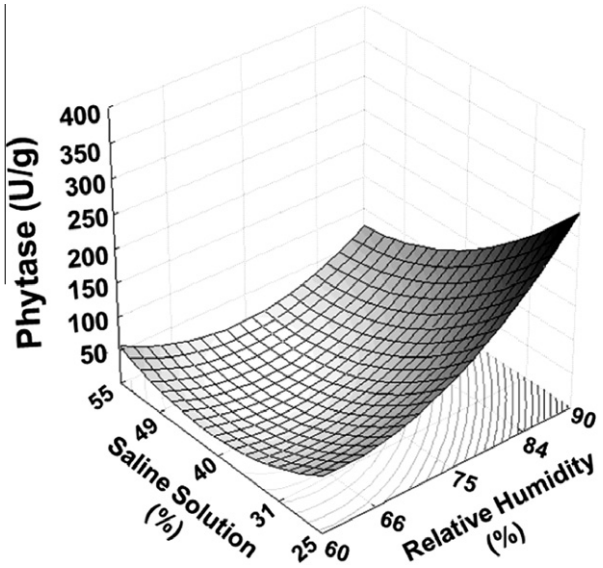

Fig. 2. A response surface representative of phytase activity as a function of relative humidity vs. the concentration of saline solution, according to the CCD.

reduced the amount of hydrolysable tannins on the castor residue extract, increasing the concentration of gallic acid monomers in the medium. Treviño-Cueto et al. (2007) studied the accumulation of gallic acid from the biodegradation of tannins present in *Larrea tridentate* leaves during fermentation with *A. niger*. The reduction of hydrolysable tannins is interesting in the improvement of the castor bean residue as an animal feed material, given that the tannins constitute anti-nutritional factors because they chelate minerals and proteins.

3.3. Ricin detection

Bands corresponding to ricin protein could be observed in unfermented castor bean residue extract (Fig. 3, lane 1). The sterilized control medium (Fig. 3, lane 2) indicated that the thermal sterilization treatment performed on the residue before inoculation was not responsible for ricin elimination.

Castor bean residue detoxification by *P. variotii* was evaluated during the course of a fermentation process that spanned 72 h, and a gradual reduction in ricin was observed over time. A reduction in the amount of ricin was observed after 24 h of fermentation; after 48 h, the ricin bands were no longer visible (Fig. 3, lanes 4 and 5).

Most likely, the fungus uses the ricin as a nutrient source, causing it to be reduced to non-detectable levels, resulting in a reduced-toxicity residue (Godoy et al., 2009).

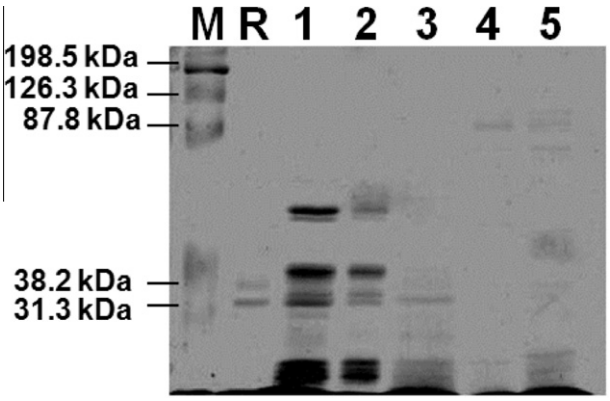

Fig. 3. SDS–PAGE of protein extracts from castor bean residue. Lane M, protein molecular weight marker; lane R, purified ricin; lane 1, unfermented substrate; lane 2, unfermented and sterilized (15′ autoclaved) substrate; lane 3, 24-h fermented substrate; lane 4, 48-h fermented substrate; lane 5, 72-h fermented substrate.

The biological detoxification of agro-industrial residues by solid-state fermentation has been previously described. Godoy et al. (2009) detoxified castor residue by fermentation with *Penicillium simplicissimum*. Joshi et al. (2011) reported the degradation of phorbol esters when *Jatropha curcas* was fermented after being deoiled by *Pseudomonas aeruginosa*. Anandan et al. (2005) tested different methods for the detoxification of castor residue ricin. Among the methods tested, only autoclaving (15 psi, 60 min) and calcium hydroxide treatment (40 g/kg castor residue) eliminated the toxin completely. Godoy et al. (2009), indicated that there were very few studies on the bio-detoxification of castor bean residue in the literature, but several physical and chemical detoxification treatments have been described, highlighting the increasing interest in the matter.

The results presented describe a simple, low-cost, biological detoxification method that converts toxic waste into potential feed material. The simultaneous production of the tannase and phytase enzymes adds additional merit to these methods.

3.4. Evaluation of cytotoxic effects induced by bio-transformed castor bean residue

The first step in evaluating the cytotoxicity of the castor bean residue extract was to screen for the minimum concentration of the extract with detectable cytotoxic effects, which was accomplished using the mitochondrial dehydrogenase activity test (MTT) under the conditions described previously (Mosmann, 1983). The minimum concentration of the castor bean residue extract that induced cellular death was 1 μg/mL; using this concentration, cell viability decreased about 70% compared to the control. Low concentrations of extract did not differ statistically from the control, indicating they were non-toxic to the cells.

To further evaluate the cytotoxicity of the castor bean residue extracts that were obtained 24, 48 and 72 h after fermentation by *P. variotii*, the extracts were incubated with RAW cells at concentrations up to 1 μg/mL, under the same conditions described above. The results of the MTT assay conducted using these extracts, as well as for the unfermented (*in natura*) castor bean residue extracts, are shown in Fig. 4. The progressive increase in cellular viability, starting from the castor bean residue extract *in natura* and proceeding through the later periods of the fermentation process, reaching almost 100% after 72 h of fermentation, was clearly observed. These results confirm the detoxification effects observed during electrophoresis analysis in the form of decreasing ricin bands.

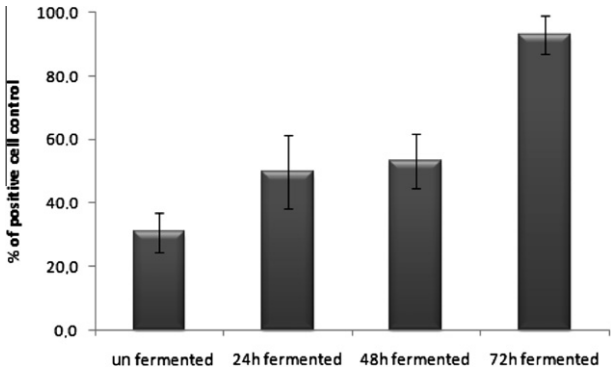

Fig. 4. Effect of unfermented castor bean residue extract on RAW cells 24, 48 and 72 h after fermentation.

4. Conclusions

The main advantage of the process described in this manuscript is the possibility to generate a feed-grade supplement based on a detoxified castor bean residue enriched with tannase and phytase. As discussed, tannase and phytase have synergistic effects, which decrease anti-nutritional factors (tannins and phytates) in animal feed.

This process has the potential to be very important for the agro-industry in Brazil because castor bean residue will be an abundant toxic residue as a byproduct of the biodiesel industry and because there is an emerging market for feed ingredients.

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