ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 09, Issue, 09, pp. 29843-29848, September, 2019

REVIEW ARTICLE OPEN ACCESS

THE GENUS *FUSARIUM* AS SOURCE OF THE ANTI-LEUKEMIC ENZYME L-ASPARAGINASE: ADJUSTMENT OF THE PRODUCTIVE CONDITIONS

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ARTICLE INFO

Article History:
Received 20th June, 2019
Received in revised form
13th July, 2019
Accepted 17th August, 2019
Published online 30th September, 2019

Key Words:
L-asparaginase; Filamentous fungi, Anti-leukemic, Factorial planning.

ABSTRACT

The enzyme L-asparaginase is used to avoid acrylamide formation in food, and as a pharmaceutical to treat Acute Lymphoblastic Leukemia. As fungi are eukaryote and good enzyme producers, it is desirable to obtain a fungal L-asparaginase as an alternative to the bacterial enzymes that are currently used in the clinical practice. Glycerol use as carbon source for the metabolism of filamentous fungi is of great interest, once it can be used as a cheaper substrate for enzyme production, L-asparaginase alike, avoiding its discard in nature. As objective of this work, we verified the potential of a strain of *Fusarium* sp. to convert glycerol to L-asparaginase. Statistical planning of bioprocesses was used to determine significant variables that could be applied for the improvement of the production. The concentrations of glycerol, L-asparagine, and the pH presented significant positive effects. By combining data from multivariate analysis, it was possible to greatly increase enzyme production. The high enzyme activity obtained (29.08 IU) is promising and opens as perspective the possibility of evaluating its anticarcinogenic activity in different tumor cell lines and, thus, to acquire a better comprehension of the L-asparaginase produced by filamentous fungi.

Citation: Suedali Villas Bôas Coelho and Ana Paula de Figueiredo Conte Vanzela. 2019. "The genus Fusarium as source of the anti-leukemic enzyme l-asparaginase: adjustment of the productive conditions", *International Journal of Development Research*, 09, (09), 29843-29848.

INTRODUCTION

L-asparaginase (L-asparagine amidohydrolase, E.C. 3.5.1.1) catalyzes the irreversible conversion of L-asparagine in L-aspartate and ammonia in aqueous environment (Shanthipriya *et al*., 2015). It has become prominent by its various and important applications, like in the food industry where it is used to reduce the levels of the carcinogenic compound acrylamide when food is processed at high temperatures (Cachumba *et al*., 2016). In the pharmaceutical industry, it is one of the main therapeutic enzymes from microbial origin used to treat proliferative disorders of the lymphoid system, mainly acute lymphoblastic leukemia (ALL), Hodgkin's lymphoma, and other lymphoid malignancies (Einsfeldt *et al*., 2016). According to Grigoropoulos *et al*. (2013), ALL is the most frequent cancer in children, and its global incidence is about 3 *per* 100.000 inhabitants, mostly affecting children under 6 years of age. Thus, L-asparaginase has been fundamental in chemotherapy protocols since more than four decades ago (Zuo *et al*., 2015).

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While normal cells can synthesize L-asparagine, tumor cells require this amino acid in abundance to guarantee rapid growth (Luhana *et al*., 2013). Thus, L-asparaginase is selective for leukemic cells, once, when administered intravenously, it hydrolyzes L-asparagine in the blood and affects only the viability of the cancer cells (Ali *et al*., 2016). This enzyme is classified by the World Health Organization as one of the most important medicines for the basic health system (Shanthipriya *et al*., 2015). Animals and plants can produce L-asparaginase, but microorganisms are its main source (Deshpande *et al*., 2014). Several bacteria are able to produce the enzyme: *Pseudomonas fluorescens*, *Serratia marcescens*, *Escherichia coli, Erwinia carotovora*, *Proteus vulgaris*, *Streptomyces karnatakensis, Streptomyces venezuelae,* (Cachumba *et al*., 2016*)*. The commercial formulations currently used are produced by *Escherichia coli and Erwinia carotovora* or *Erwinia chrysanthemi*. However, these can cause hypersensitivity and toxicity (Mahajan *et al*., 2013). Other producers like filamentous fungi and yeasts have been progressively studied in depth (Loureiro *et al*., 2012). Hosamani and Kaliwal (2011) observed that eukaryote microorganisms, including yeasts and filamentous fungi from the genera *Aspergillus*, *Penicillium* and *Fusarium*, produce L-

asparaginase with less adverse effects. In fact, a fungal L-asparaginase decreased viability of leukemic cells without toxicity for normal cells *in vitro* (Loureiro *et al*., 2012). The large production of biodiesel in the last years brought glycerol as main byproduct, in such a way that to avoid problems with its discard, it is increasingly needed to search alternative applications (Rivaldi *et al*., 2008). Filamentous fungi like *Fusarium* sp. T22.2, a strain selected in previous studies, are able to convert glycerol to value-added products like L-asparaginase, contributing to decrease the costs of production and helping to solve the problem of glycerol discard in nature (Gonçalves *et al*., 2016). Several factors are important for the industrial production of L-asparaginase, either to increase yield or to improve the economic viability. The carbon and nitrogen sources, their concentrations, pH, aeration, temperature, time of fermentation, and especially the microbial agent strongly influence the productive process (Baskar and Sahadevan, 2012). The most used carbon sources are glucose, starch, and maltose, which can be replaced by glycerol for microorganisms that can do the bioconversion (Cachumba *et al*., 2016). Solid state fermentation is used to produce L-asparaginase, but submerged fermentation remains the main productive process (Lopes *et al*., 2015). As a main point for the production, the search for new strains needs to be taken into account, seeking for enzymes with better therapeutic properties and less toxic effects (Dutta *et al*., 2015). Thus, the objective of this work was to verify the potential of a strain of *Fusarium* sp. to convert glycerol to L-asparaginase.

MATERIAL AND METHODS

Strain maintenance: *Fusarium* sp. T22.2 was cultivated weekly in Czapek-Dox agar (Frisvad and Samson, 2004) at 30 °C to collect spores for inoculums. For strain maintenance, small blocs of agar covered by mycelium were submerged in sterile distilled water contained in 2 mL micro tubes, and stored under refrigeration (4 ± 1 ºC). Stocks were replaced at every 6 months.

Factorial planning (multivariate analysis): Experimental conditions for productive bioprocesses were generated with *Statistica* 7.0. (StatSoft Inc.) The concentration of glycerol (carbon source), L-asparagine (nitrogen source), and the pH were used as independent variables in a 2^n full factorial planning, where *n* is the number of variables studied, yielding 8 different runs. A fractional factorial planning 2^{n-1} or 2^{n-2} would be applied instead of a full planning if the number of independent variables to be analyzed were too large, making the experimental runs excessively numerous. However, as three variables were investigated in the present study, a full factorial planning with 8 runs was preferable. The minimal and maximal levels of each variable were: 0.5 and 20 g L^{-1} for glycerol; 0.5 and 10 g L^{-1} for L-asparagine; 5.5 and 6.5 for pH. Production of L-asparaginase activity was investigated at 24 h intervals from 48 to 120 h. Statistical analysis was performed to identify the best level of each variable and its interactions in order to apply for process improvement. All reagents for medium preparation and enzyme determination were of analytical grade.

L-asparaginase production by liquid state fermentation: The bioprocesses were conducted by liquid state stationary fermentation in 60 mL of media contained in 125 mL Erlenmeyer flasks. Besides glycerol, L-asparagine and pH adjusted as determined by factorial planning, media contained: 2 g L^{-1} KH_2PO_4; 0.5 g L^{-1} NaCl; 0.2 g L^{-1} $MgSO_4$ $7H_2O$. Spores from strain T22.2 suspended in distilled water were inoculated to a final concentration of 10^5 per mL of media. Cultures in triplicate were incubated for 120 h at 30 ºC. Samples were removed at 24 h intervals from 48 h of incubation, and centrifuged for 10 minutes at 3.000 g. Enzyme activity was determined in the supernatant.

Improvement of L-asparaginase production: After factorial planning and statistical analysis, other bioprocesses were elaborated by combining different concentrations of glycerol and L-asparagine, and by adjusting the pH according to the effects determined for each variable and their interactions. Three new media were elaborated and contained: A) 25 g L^{-1} glycerol; 5 g L^{-1} L-asparagine; pH 8; B) 29,7 g L^{-1} glycerol; 10 g L^{-1} L-asparagine; pH 7; C) 5 g L^{-1} glycerol; 15 g L^{-1} L-asparagine; pH 6. Other medium constituents (KH_2PO_4; NaCl; $MgSO_4$ $7H_2O$) did not vary. Bioprocesses were inoculated and conducted by liquid state stationary fermentation as described above. Cultures were incubated for 120 h at 30 ºC, and samples were removed daily from 72 h for determination of L-asparaginase activity.

Determination of L-asparaginase activity: L-asparaginase was determined as adapted from Chow and Ting (2015). The reaction mixture was prepared in 1.5 mL polypropylene tubes by mixing 0.1 mL of Tris. HCl 1 mol L^{-1} pH 7 with 0.2 mL of L-asparagine 8 mmol L^{-1} and 0.1 mL of distilled water. After pre-incubation at 37 ºC, 0.1 mL of the supernatant containing the enzyme was added, and the reaction was incubated for 37 ºC for 30 minutes. Enzyme reaction was stopped with 0.1 mL of trichloroacetic acid (TCA) 1.5 mol L^{-1}. Control samples were prepared by addition of TCA before the enzyme, and considered to be the time zero of reaction. An aliquot of 0.1 mL was collected from the reaction and added to 0.45 mL of Nessler reagent plus 0.6 mL of distilled water to determine the amount of ammonia formed by L-asparagine hydrolysis. After incubating at 28 ºC for 15 minutes, absorbance was determined at 450 nm against a blank prepared as the reaction, except for replacing the supernatant by distilled water. The concentration of ammonia liberated in the reaction was calculated against a calibration curve, considering the difference among the final (30 minutes of reaction) and the initial (time zero) absorbance. L-asparaginase activity was determined in international units (IU); whereas one unit is the amount of enzyme which forms 1 µmol min^{-1} mL^{-1} of ammonia in the reaction conditions.

RESULTS AND DISCUSSION

Factorial planning (multivariate analysis) and L-asparaginase production: Filamentous fungi are able to produce L-asparaginase, a therapeutic enzyme used to treat diseases like ALL and other disorders of the lymphoid system. Thus, after studies of Gonçalves *et al*. (2016), which verified that *Fusarium* sp. T22.2 produced this enzyme with an activity that liberated 11.4 µg min^{-1} mL^{-1} of ammonia in the reaction (equivalent to approximately 0.7 IU), we decided to improve the productive process. Due to the augment of biofuel production in Brazil, including biodiesel, there has been a large production of biodiesel-derived glycerol, which corresponds to 10% of the production (Papanikolaou and Aggelis, 2002). There is an excess of glycerol being produced, what demands search for viable applications as a way to avoid its discard in nature. Unlike most of the reported studies, in the present work, we used glycerol as carbon source for the

production of L-asparaginase by *Fusarium* sp. T22.2. By applying factorial planning we were able to generate 8 different bioprocesses combining the maximal and minimal values of the independent variables analyzed (glycerol and L-asparagine concentrations and pH) in a multivariate analysis. The main purpose of this approach is to avoid the excessive number of steps usually taken to improve a productive process when univariate analysis is used. Besides, analysis of each variable at once, while others are fixed until the best value of each parameter is achieved, presents as disadvantage the impossibility to determine the interactions among variables, what could be done with the approach we used herein. Determination of L-asparaginase in the supernatant of cultures showed that after 48 h almost no activity is produced by *Fusarium* sp. T22.2. For this reason, results of L-asparaginase production in the planned bioprocesses are shown from 72 h, when the enzymatic activity starts to increase (Table 1). The maximal activity was obtained after 96 h, in bioprocess 8 and reached 27.33 IU as shown in Table 1. This process – supplemented with glycerol at 20 g L^{-1}; L-asparagine at 10 g L^{-1}; and adjusted to pH 6.5 – was the best condition for the enzyme production, and allowed to obtain a high activity even after 72 h (10.34 IU). Other bioprocesses also yielded good activities, although at higher times of incubation.

Bioprocesses 3 and 4 induced the production of 8.18 IU and 14.59 IU of L-asparaginase after 120 h of cultivation (Table 1). Statistical analysis of data allowed determination of the effects of the independent variables (glycerol, L-asparagine, and pH) and their interactions. Effects are shown by means of Pareto graphs for the response L-asparaginase activity after 72 h (Graph 1) and 96 h (Graph 2). Analysis of variance is shown in Table 2. For significance determination, p-value threshold was equal to 0.05. Effects were statistically significant, as shown by p-values < 0.05 (Table 2), whereas interaction among variables 1 and 3 (glycerol by pH) in the cultures of 120 h was not significant (p = 0.08). In Pareto graphs, significance of effects is shown by the p-value threshold dotted line, set to 0.05. Significant effects extend beyond this line to the right, while non-significant ones remain at left (Graphs 1-3). L-asparaginase production after 72 h of cultivation was strongly and positively influenced by the concentration of L-asparagine, which showed the highest effect among the variables analyzed (6.47), followed by glycerol concentration (4.63) and pH (2.82). Such positive effects indicate that the highest level of each variable should be applied to improve enzyme production, i.e. L-asparagine at 10 g L^{-1} should be applied instead of 0.5 g L^{-1}. Similarly, glycerol supplementation at 20 g L^{-1} may yield a better enzyme production than 0.5 g L^{-1}.

Table 1. Production of L-asparaginase by *Fusarium sp.* T22.2 in bioprocesses designed by factorial planning. Processes were designed by the combination of the minimum and maximal levels of glycerol, L-asparagine, and pH. Enzyme activity was determined in the supernantant, and is shown as mean ± standard deviation of three replicates. One unit was defined as the amount of enzyme which catalyzes the formation of 1 µmol of ammonia per mL per minute of reaction at 37 °C and pH 7. IU international units

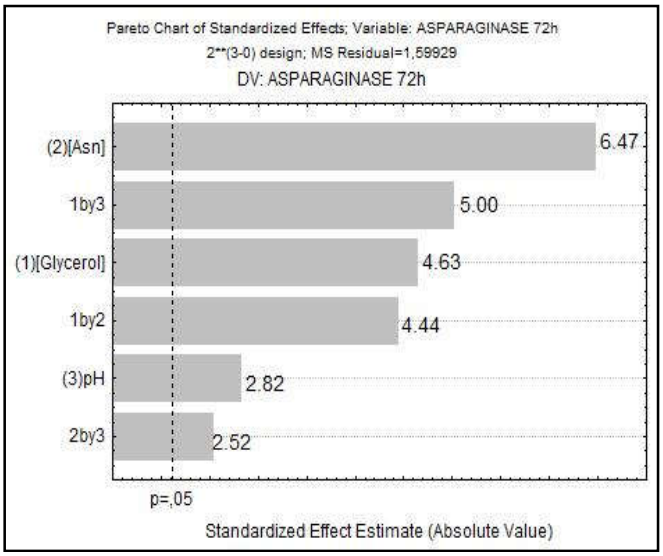
Bioprocess	Glycerol (g L^{-1})	Asparagine (g L^{-1})	pH	L-asparaginase Activity (IU)		
				72 h	96 h	120 h
1	0.50	0.50	5.50	1.32±0.82	1.49±0.13	1.49±0.22
2	20.00	0.50	5.50	0.75±0.07	1.56±0.08	0.55±0.15
3	0.50	10.00	5.50	2.99±0.08	2.69±0.33	8.18±1.01
4	20.00	10.00	5.50	3.19±0.27	4.65±0.88	14.59±1.06
5	0.50	0.50	6.50	0.81±0.02	0.73±0.06	0.37±0.23
6	20.00	0.50	6.50	1.89±0.61	0.94±0.11	1.08±0.11
7	0.50	10.00	6.50	1.25±0.40	1.79±0.62	3.17±0.05
8	20.00	10.00	6.50	10.34±1.22	27.33±1.71	3.87±1.08

Table 2. Analysis of Variance (ANOVA) on data obtained from bioprocess designed by factorial planning for L-asparaginase production

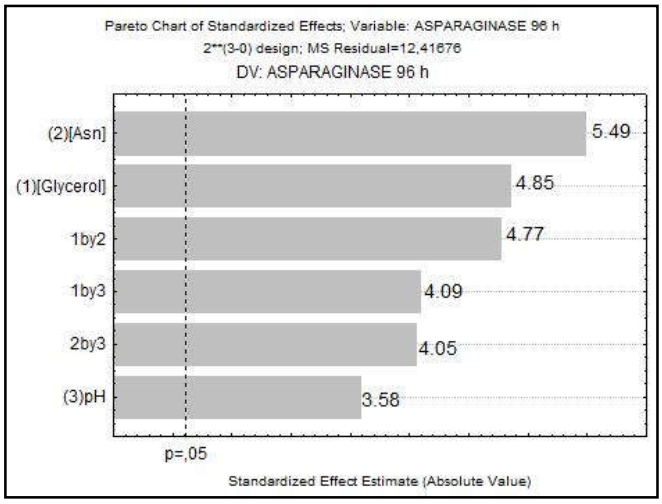
Variable: L-asparaginase (72 h); R-square 0.87806					
Factor	SS	df	MS	F	p
(1) [Glycerol]	34.2918	1	34.29177	21.44186	0.000239
(2) [Asn]	67.0254	1	67.02544	41.90949	0.000006
(3) pH	12.7219	1	12.72195	7.95475	0.011788
1by2	31.5199	1	31.51991	19.70869	0.000359
1by3	40.0226	1	40.02261	25.02523	0.000109
2by3	10.1965	1	10.19646	6.37561	0.021802
Error	27.1879	17	1.59929		
Total SS	222.9661	23			
Variable: L-asparaginase (96 h); R-square 0.87811					
Factor	SS	df	MS	F	p
(1) [Glycerol]	292.473	1	292.4734	23.55472	0.000149
(2) [Asn]	374.188	1	374.1880	30.13571	0.000040
(3) pH	159.211	1	159.2106	12.82223	0.002303
1by2	282.744	1	282.7436	22.77112	0.000177
1by3	207.886	1	207.8864	16.74239	0.000760
2by3	204.124	1	204.1236	16.43936	0.000824
Error	211.085	17	12.4168		
Total SS	1731.711	23			
Variable: L-asparaginase (120 h); R-square 0.94545					
Factor	SS	df	MS	F	p
(1) [Glycerol]	17.1813	1	17.1813	10.3217	0.005106
(2) [Asn]	260.8980	1	260.8980	156.7345	0.000000
(3) pH	100.8539	1	100.8539	60.5880	0.000001
1by2	20.8036	1	20.8036	12.4977	0.002542
1by3	5.5574	1	5.5574	3.3386	0.085280
2by3	85.1956	1	85.1956	51.1813	0.000002
Error	28.2980	17	1.6646		
Total SS	518.7878	23			

Data also showed that pH near the neutrality is better than an acidic value for enzyme production after 72 h. However, among these variables, the most important was L-asparagine concentration. Yet, some variable interactions were more important than the effect of the variable alone. For instance, interaction 1 by 3 (glycerol concentration and pH) presented an effect of 5 on enzyme production, higher than the isolated effects of glycerol and pH. The positive value for interaction 1 by 3 means that at higher concentrations of glycerol, a higher value of pH must be applied to improve L-asparaginase production after 72 h (Graph 1). As interactions 1 by 2 (glycerol concentration and L-asparagine concentration) and 2 by 3 (L-asparagine concentration and pH) also presented positive effects for enzyme production, the adjustment of the production was facilitated, because these demand that increases in one variable level be followed by increases in the others, obeying the individual effects which indicated that the highest level of each variable should be applied (Graph 1).

A highly significant positive effect of L-asparagine concentration on L-asparaginase production by *Fusarium* sp. T22.2 after 96 h of cultivation was also determined (Graph 2). This effect (5.49) was similar to the effect determined for this variable on enzyme production after 72 h (6.47). However, after 120 h of cultivation, the concentration of L-asparagine had its higher effect (12.52) on enzyme production (Graph 3), indicating that for longer times of cultivation, the concentration of this nitrogen source, which might possibly act as inducer of the gene coding for L-asparaginase, should be higher when the process is started because it is progressively depleted in the medium. Glycerol effects on enzyme production were also similar after 72 h (4.63) and 96 h (4.85), as shown in Graphs 1 and 2. However, glycerol effect decreased after 120 h of cultivation (3.21) as shown in Graph 3, indicating that it performed a role different of L-asparagine for the productive process. The role of glycerol, as carbon source, might have been to sustain growth of the strain, what could be more important at the initial times of cultivation than at the final times, when the producing biomass may have already been formed.

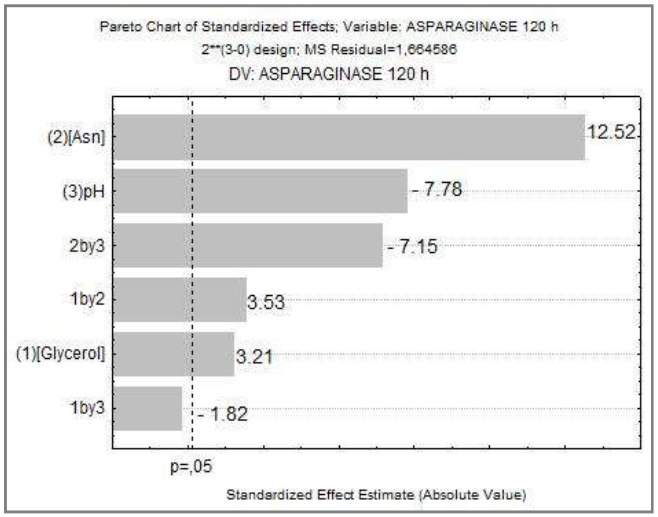


Graph 1. Pareto graph showing value and statistical significance of the effects of [Asn] (L-asparagine concentration); [Glycerol] (glycerol concentration), pH, and interactions among these independent variables for L-asparaginase production by *Fusarium* sp. T22.2 after 72 h of cultivation. Interactions: 1 by 3, glycerol by pH; 1 by 2, glycerol by L-asparagine; 2 by 3, L-asparagine by pH



Graph 2. Pareto graph showing value and statistical significance of the effects of [Asn] (L-asparagine concentration); [Glycerol] (glycerol concentration), pH, and interactions among these independent variables for L-asparaginase production by *Fusarium* sp. T22.2 after 96 h of cultivation. Interactions: 1 by 3, glycerol by pH; 1 by 2, glycerol by L-asparagine; 2 by 3, L-asparagine by pH

The most striking change in the variable behavior for the enzyme production was that of pH, which turned to be negative after 120 h of cultivation, as well as of its interaction with L-asparagine concentration 2 by 3 (Graph 3). This indicates that for longer periods of cultivation, the adjustment of the initial pH of media at the lower level evaluated (pH = 5.5) is better for enzyme production. Analyzing in function of the cultivation time, it means that the higher level of pH (pH = 6.5) – or pH near the neutrality – tends to induce a more rapid production than the lower value, anticipating the maxima of enzyme activity.



Graph 3. Pareto graph showing value and statistical significance of the effects of [Asn] (L-asparagine concentration); [Glycerol] (glycerol concentration), pH, and interactions among these independent variables for L-asparaginase production by *Fusarium* sp. T22.2 after 120 h of cultivation. Interactions: 1 by 3, glycerol by pH; 1 by 2, glycerol by L-asparagine; 2 by 3, L-asparagine by pH

Considering the effects determined for the variables and their interactions, as well as the highest enzyme activity obtained after 96 h, it was concluded that values similar to highest level of each variable would be beneficial for the production. To test

this hypothesis, three conditions were used for production, whose results are shown in Figure 1.

Improvement of L-asparaginase production: In the first condition developed for L-asparaginase production, bioprocess A, The values for glycerol and pH were above the highest level utilized in the planned bioprocesses, while L-asparagine was adjusted to an intermediary value. This condition induced production of 15.66 IU of L-asparaginase, but the time to obtain the maximal production in the culture was delayed to 120 h (Figure 1).

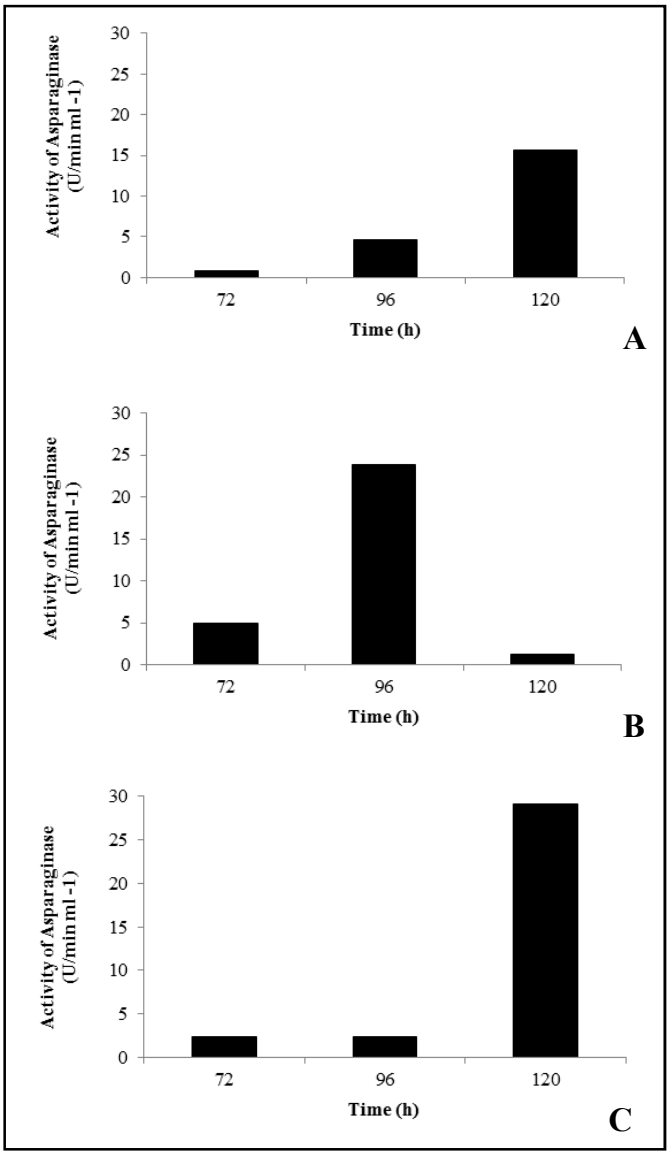


Figure 1. Production of L-asparaginase by liquid state fermentation. Cultures of *Fusarium* sp. T.22.2 were prepared in three media designed as follows: Bioprocess A (25 g L^{-1} glycerol; 5 g L^{-1} L-asparagine; pH 8.0); Bioprocess B (29.7 g L^{-1} glycerol; 10 g L^{-1} L-asparagine; pH 7.0) ; Bioprocess C (5 g L^{-1} glycerol; 15 g L^{-1} L-asparagine; pH 6.0). Media were inoculated with 10^5 conidia per mL, and incubated at 30 °C. Activity of L-asparaginase was determined in the supernatant of cultures, considering the µmoles of ammonia liberated in a thirty minute reaction at pH 7.0 and 37 °C. Enzyme activity was expressed in international units (IU).

Enzyme production at lower values of L-asparagine would be preferable because this substrate is expensive. Although production was delayed to 120 h, the enzyme activity produced in Bioprocess A was still high: 15.66 IU (Figure 1). Hosamani *et al*. (2011) evaluated different carbon sources for L-asparaginase production by *Fusarium equiseti* in solid state fermentation: it was produced 3.26 U/mL and 0.21 U/mL in fermentations performed with soy flour and sugarcane bagasse. Deshpandes *et al*. (2014) utilized *Streptomyces ginsengisoli* to produce L-asparaginase by submerged fermentation in media supplemented with sucrose, glucose, lactose and manitol, and after optimization obtained 3.23 µmol/mL of L-asparaginase.

Cultivation of *Fusarium* sp. T22.2 in the conditions of Bioprocess B (Figure 1) elevated production of L-asparaginase in comparison to that obtained in Bioprocess A. By adjusting the medium with glycerol at 29.7 g L^{-1}; L-asparagine at 10 g L^{-1}; and pH 7.0, it was possible to obtain 23.92 IU of L-asparaginase after 96 h. A shorter time of production is also preferable to longer ones. The enzyme activity produced in Bioprocess B was slightly lower than that produced in Bioprocess 8 (Table 1), perhaps due to the very high concentration of glycerol used (29.7 g L^{-1}). The best condition for L-asparaginase production by *Fusarium* sp. T22.2 was Bioprocess C, where the enzyme activity reached 29.08 IU after 120h. In this condition, pH was adjusted to 6.0, a value a little lower than the pH 6.5 indicated as preferable for L-asparaginase production by factorial planning. L-asparagine was adjusted to 15 g L^{-1}, a higher value than indicated by the statistical analysis (10 g L^{-1}) and glycerol was adjusted to 5 g L^{-1}, 10 times more than the minimum level applied in the planned bioprocesses (0.5 g L^{-1}) but 3 times less than the maximum level (20 g L^{-1}).

All the three bioprocesses A, B, C designed by considering statistical analysis and the effects determined for glycerol and L-asparagine concentration, pH, and the effects of the variable interactions (Graph 1, Graph 2), showed to be good conditions for L-asparaginase production by *Fusarium* sp. T22.2. By means of this approach, we got to elevate the production by approximately 40 times compared to that reported in the initial studies with strain T22.2 (Gonçalves *et al*., 2016). Einsfeldt *et al*. (2016) produced recombinant L-asparaginase from *Zymomonas mobilis* expressed in *E. coli*, reaching an extracelular activity of 0.13 U/mL. In a work where several bacterial isolates from soil were tested for L-asparaginase production, *Streptococcus* spp. D1 produced 11.6 U (Wakil and Adelegan, 2015). Vachhani and Desai (2018) also isolated several bacteria from soil and the best isolated produced 18.66 U of L-asparaginase after 96 h of cultivation in tryptone glucose yeast extract with 1% L-asparagine. In another work, *Pseudomonas pseudoalcaligenes* JHS-71 produced 240 U (Badoei-Dalfard, 2016). These studies show several bacterial isolates producing L-asparaginase, but, once the bacterial enzymes employed in commercial formulations are usually implicated in allergic responses, it would be suitable to investigate if the eukaryote enzymes, like those obtained from fungi, may present less adverse effects. To support investigation of fungal L-asparaginase concerning to its therapeutic or toxic properties, presence or absence of the secondary activity of L-glutaminase – as inherent of several bacterial forms of the enzyme – and for further studies of kinetics, it is important to increase production to suitable activities, like done in the present work.

Conclusion

The present work showed the suitability of a strain of *Fusarium* as eukaryote source of L-asparaginase. The maximum enzyme activity obtained – approximately 29 IU – was higher than reported in several works, and encourages

further studies for enzyme purification, kinetics determination, and evaluation of anti-leukemic properties. Besides, the enzyme activity was extracellular and could be maximized from 72 to 120 h, what characterizes a short time of production, comparable to that of bacterial bioprocesses. As an advantage, while several bioprocesses for L-asparaginase production requires, besides the enzyme inducer L-asparagine, carbon sources like glucose, sucrose, or others of higher cost, the strain *Fusarium* sp. T22.2 used in the present study produced L-asparaginase in media supplemented with glycerol, an alternative carbon source whose biotechnological exploitation is important from the ecological and economical perspectives.

Acknowledgements

This work is part of the research conducted by S.V.B.C.B. in partial fullfillment of the requirements for the doctor degree, under the Program of Post-graduation in Biofuels of UFVJM.

REFERENCES

Ali, U. Naveed, M., Ullah, A., Ali, K., Shah, S. A., Fahad, S., Mumtaz, A., S. 2016. L-asparaginase as a critical component to combat Acute Lymphoblastic Leukaemia (ALL): A novel approach to targ*et al*L. Eur J Pharmacol. 771: 199-210.

Badoei-Dalfard, A. 2016. L-asparaginase production in the *Pseudomonas pseudoalcaligenes* strain JHS-71 isolated from Jooshan Hot-spring. Mol. Biol. Res. Commun. 5: 1-10.

Baskar, G. and Sahadevan, R. 2012. Optimization of Culture conditions and Bench-Scale: Production of L-asparaginase by submerged Fermentation of *Aspergillus terreus* MTCC 1782. J. Microiol. Biotechnol. 22: 923–929.

Cachumba, J. J. M., Antunes, F. A. F., Peres, G. F. D., Brumano, L. P., Santos, J. C., Silva, S. S. 2016. Biotechnology and industry microbiology current applications and different approaches for microbial L-asparaginase production. Braz. J. Microbiol. 47: 77-85.

Chow, Y. and Ting, A. S. Y. 2015. Endophytic L-asparaginase-producing fungi from plants associated with anticancer properties. J. Adv. Res. 6: 869–876.

Desphande, M., Choubey, P., Agashe, M. 2014. Studies on optimization of growth parameters for L-asparaginase: production by *Streptomyces ginsengisoli.* Scientific. World Journal. 2014: 895-167.

Dutta, S., Ghosh, S., Pramanik, S. 2015. L-asparaginase and L-glutaminase from *Aspergillus fumigatus* WL002: Production and Some Physicochemical Properties. Appl. Biochem. Microbiol. 51: 425–431.

Einsfeldt, K., Baptista, I. C., Castanheira, J. C., Pereira, V., Costa-Amaral, I. C., Costa, E. S., Almeida, R. V. 2016. Recombinant L-asparaginase from *Zymomonasmobilis*: A Potential New Antileukemic Agent Produced in *Escherichia coli*. *PLoS ONE.* 11.

Eisele, N., Linke, D., Bitzer, K., Na`amnieh, S., Nimtz, M., Berger, R. G. 2011. The first characterized asparaginase from a basidiomycete, *Flammulina velutipes*. Bioresour. Technol. 102: 3316-3321.

Frisvad, J. C. and Samson, R. A. 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*: A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Studies in Mycology. 49: 1–174.

Gonçalves, A. B., Maia, A. N. F., Rueda, J. A., Vanzela, A. P. F. C. 2016. Fungal production of the anti-leukemic enzyme L-asparaginase: from screening to medium development. Acta Sci., Biol. Sci. 38: 387-394.

Grigoropoulos, N. F., Petter, R., 'T Veer, M. B. V., Scott, M. A., Follows, G. A. 2013. Leukaemia update. Part 1: diagnosis and management. BMJ Carrers. 346.

Hosamani, R., and Kaliwal, B. B. 2011. Isolation, molecular identification and optimization of fermentation parameters for the production of L-asparaginase, an anticancer agent by *Fusarium equiseti*. I. J. M. R. 3: 108-119.

Lopes, A. M., Oliveira-Nascimento, L., Ribeiro, A., Tairum Jr, C. A., Breyer, C. A., Oliveira, M. A.,... Pessoa, A. 2015. Therapeutic L-asparaginase: upstream, downstream and beyond. Crit. Rev. Biotechnol. 37: 82-99.

Loureiro, C. B., Borges, K. S., Andrade, A. F., Tone, L. G., Said, S. 2012. Purification and Biochemical Characterization of Native and Pegylated form of L-asparaginase from *Aspergillus terreus* and Evaluation of Its Antiproliferative. Activity. Adv. Microbiol. 2: 138-145.

Luhana, K. K., Dave, A. A., Patel, K. U. 2013. Production, Purification & Characterization of Extracellular L-asparaginase (Anti Cancerous Enzyme) from *Aspergillus niger*. International Journal of Chemtech Applications, 2: 14-25.

Mahajan, R. V., Saran, S., Saxena, R. K. Srivastava, A. K. 2013. A rapid, efficient and sensitive plate assay for detection and screening of L-asparaginase-producing microorganisms. FEMS Microbiology Letters. 341: 122–126.

Papanikolaou, S., and Aggelis, G. 2002. Lipid production by *Yarrowia lipolytica* growing on industrial glycerol in a single-stage continuous culture. Bioresour. Technol. 2: 43-49.

Rivaldi, J. D., Sarroub, B. F., Fiorilo, R., Silva, S. S. 2008. Glicerol de biodiesel: Estratégias biotecnológicas para o aproveitamento do glicerol gerado da produção de biodiesel. Revista Biotecnologia Ciência & Desenvolvimento. 37.

Shanthipriya, A., Koteswara, V. R., Girisham, S., Reddy, S. M. 2015. Factors influencing L-asparaginase Production by Three Thermophilic Caprophilous Fungi. *Int. J. Pharma Sci.,* 5: 1242–1248.

Vachhani, J. and Desai, B. 2018. Isolation, identification and production of L-asparaginase producing bacteria from soil. BMR Microbiology. 4: 1-6.

Wakil, S. M., and Adelegan, A. A. 2015. Screening, production and optimization of L-asparaginase from soil bacteria isolated in Ibadan, South-wertern *Nigerria. Journal of Basic & Applied Sciences*. 11: 39-51.

Zuo, S., Zhang, T., Jiang, B., Mu, W. 2015. Recent research progress on microbial L-asparaginases. Appl. Microbiol. Biotechnol. 99: 1069-1079.

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