XII Seminário Brasileiro de Tecnologia Enzimática
ENZITEC 2016

Lipase Production by *Geotrichum candidum* on Semi-Solid State Fermentation Using Corn Bran

Sílvio Aparecido Melquides[1], Elizama Aguiar-Oliveira[2], Guilherme Sousa Silva[3], Eliana Setsuko Kamimura[3], Rafael Resende Maldonado[1,4,5]

[1]Faculdade Municipal Professor Franco Montoro (FMPFM), Mogi Guaçu, São Paulo, Brasil.
[2] Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia (IMS-CAT/UFBA), Vitória da Conquista, Bahia, Brasil.
[3]Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo (FZEA/USP), Pirassununga, São Paulo, Brasil.
[4]Colégio Técnico de Campinas, Universidade Estadual de Campinas (COTUCA/UNICAMP), Campinas, São Paulo, Brasil.
[5] Instituto Federal de São Paulo (IFSP), Boituva, São Paulo, Brasil.
E-mail: ratafta@yahoo.com.br.

ABSTRACT

Agro-industrial wastes as substrates for solid state fermentation result in low cost processes and bioproducts with high value added. The aim of this study was to evaluate the production of lipase by Geotrichum candidum through a semi-solid state fermentation using corn bran (a by-product of corn processing) supplemented with NH_4Cl, corn oil and water. The results indicated that the corn bran is a feasible substrate for lipase production by a semi-solid state fermentation. The best conditions for obtaining the enzyme at 35°C were: 60 g of corn bran supplemented with NH_4Cl, corn oil and water to achieve 5% (w/w) of nitrogen, 15% (w/w) of lipid and 40% (w/w) of moisture which resulted, after 24 h, in a maximum productivity of 0.72 U g^{-1} h^{-1} and, at 48 h, in a maximum lipase activity of 29.4 U g^{-1}.

Keywords: Lipase, solid state fermentation, corn bran, corn oil, *Geotrichum*.

INTRODUCTION

Lipases (triacylglycerol acyl hydrolases EC 3.1.1.3) are enzymes of great industrial importance and they have been widely investigated due to its ability to hydrolyse (reversibly) triglycerides and to catalyze esterification, transesterification and interesterification reactions in oil/water interface. Lipase production by the fungus *Geotrichum candidum* has been investigated and it has been shown as a great potential bioprocess (Asses et al., 2009; Maldonado et al., 2012, 2014A-C). The cultivation of microorganisms and the consequential enzyme production can be performed by: submerged fermentation in a liquid medium (SMF), semi-solid (SSF) or solid state fermentation (SF). The difference between SF and SSF is the amount of free water present (Gervais & Molin, 2003). The applicability of various materials such as agro-industrial by-products of low cost makes SF and SSF very interesting, in addition, the increase in free water content can come to alleviate many of the possible drawbacks.

The production of lipases by similar technologies has also been applied with the most varied solid substrates (by products) (Amorim et al., 2013; Okino-Delgado & Fleuri, 2014;

Singh et al., 2014). Microorganisms from the genus *Geotrichum* has also been applied in different SSF processes (Canli et al., 2012; Sun et al., 2008). Due to the various applications by the SSF, the aim of this study was to evaluate and determine the best conditions for lipase production by *Geotrichum candidum* NRRLY-552 using corn bran.

MATERIAL AND METHODS

Inoculum: *Geotrichum candidum* NRRLY-552 was cultivated on Malt Agar Yeast Medium for 72 h at 30°C. A circular area (ϕ = 1 cm) from the solid medium containing the microorganism spores was used as inoculum as described before (Maldonado et al., 2014A-C). The amount of water added in each experiment varied according to the final moisture content fixed for each condition evaluated.

Semi-solid state fermentation (SSF): The substrate used, corn bran (CB) [composition % (w/w) of: 18.2% protein, 8.4% oil and 10% moisture], is a solid by-product obtained from the extraction of corn germ cake; this substrate was purchased from Ingredion (Mogi Guaçu, Brazil). In a first step, 60 g of CB was supplemented with NH_4Cl, corn oil (CO) and distilled water in order to achieve concentrations (% w/w on dry base) of 5% of total nitrogen (N), 12.5% of CO and 30% of moisture (W). After inoculation, the flasks (400 mL) were incubated (35°C) without stirring for 48 h and samples of 2 g were collected at 24 and 48 h to determine the lipase activity. On the second step, a 2^{4-1} fractional factorial design (FFD) was carried out to evaluate the independent variables at % (w/w): N (4-6%) and CO (10-15%), W (20-40%) and CB (20-60 g) (Table 2). Fermentations occurred at 35°C without stirring and 2 g of samples were collected at 24, 48 and 72 h. On the third step, experiments were performed varying only N at 6, 8 and 10% (w/w) with the other variables fixed at 15% (w/w) of CO, 40% (w/w) of W and 60 g CB. These fermentations were carried out in triplicate at 35°C without stirring, and samples of 2 g of fermented material were collected at 24, 48 and 72 h to determine the lipase activity.

Lipase activity: A titrimetric method (with oleic acid as the standard) from a mixture of Arabic gum solution (7% w/v) and olive oil at a ratio of 75:25% (v/v) was applied as described previously (Maldonado et al., 2012, 2014A-C). The amount of enzymatic activity was expressed as U g^{-1} of dry substrate.

Statistical analysis: The results from the 2^{4-1} FFD were evaluated by the analysis of variance (ANOVA) and the Tukey test at 95% of confidence using Statistica 8.0 - Statsoft®.

RESULTS AND DISCUSSION

The results in the first step with *G. candidum* cultivated in CB (data not presented) confirmed the capacity of lipase production with average productivities (U g^{-1} h^{-1}) of 0.854 (24 h) and 0.318 (at 48 h) and suggested the levels of the independent variables for the 2^{4-1} FFD performed in sequence (Table 1). From the data obtained it was possible to calculate the effect of each variable on lipase production which showed that, on average, the lipase production was higher at 24 h. After this period, only N total showed a positive and statistically significant effect (p<0.05) of 7.84 U g^{-1} and, at 48 h, in addition to the variable N, the variable CO also had a positive and statistically significant effect (p = 0.03) of 6.82 U g^{-1}. With these results, new conditions were established: for N new levels of 6, 8 and 10% (w/w); for CO its +1 level (15% w/w) was fixed due to its significant effect in 48 h; for the variables

W and CB, their conditions were fixed based on the best lipase activity (LIP) (run 8, from Table 1); the results of this step are shown in Table 2.

Table 1. Matrix for 2^{4-1} FFD for lipase production by *G. candidum* using corn bran in SSF.

Run	N (% w/w)	CO (% w/w)	W (% w/w)	CB (g)	LIP (U g^{-1})*		
					24 h	48 h	72 h
1	4.0(-1)	10(-1)	20(-1)	20 (-1)	8.29	7.09	2.27
2	6.0 (+1)	10 (-1)	20 (-1)	60 (+1)	11.86	3.46	9.46
3	4.0(-1)	15(+1)	20 (-1)	60 (+1)	5.84	5.84	0.00
4	6.0 (+1)	15(+1)	20 (-1)	20 (-1)	11.79	14.18	7.03
5	4.0(-1)	10 (-1)	40 (+1)	60 (+1)	8.15	3.99	3.99
6	6.0 (+1)	10 (-1)	40 (+1)	20 (-1)	16.37	10.85	6.72
7	4.0(-1)	15(+1)	40 (+1)	20 (-1)	5.32	9.42	5.32
8	6.0(+1)	15(+1)	40 (+1)	60 (+1)	18.92	21.64	16.19
9	5.0 (0)	12.5 (0)	30 (0)	40 (0)	11.42	7.57	5.00
10	5.0 (0)	12.5 (0)	30 (0)	40 (0)	10.13	5.00	5.00
11	5.0 (0)	12.5 (0)	30 (0)	40 (0)	8.85	6.28	7.57

Table 2. Influence of total nitrogen composition (N) (% w/w) on lipase production (LIP) by *G. candidum* using corn bran in SSF.

N (% w/w)	LIP (U g^{-1})*		
	24 h	48 h	72 h
6	9.2 ± 2.1	12.4 ± 3.6	7.4 ± 3.9
8	6.9 ± 1.4	11.0 ± 2.7	6.9 ± 3.6
10	17.3 ± 4.1	29.4 ± 2.1	26.2 ± 3.4

To compare the three conditions evaluated (Table 2), the analysis of variance was conducted at 48 h of fermentation and it was observed a significant difference (p<0.01) among the three evaluated N values and, also, no significant difference among the replicates performed for each condition (p=0.93). A Tukey test determined the honestly significant difference (HSD), with p<0.05, as 10.02, indicating that the conditions of N6% and N8% were statistically equal (difference of average=1.4) and N10% condition is statistically different from N6% and N8% (difference of average of 17 and 18.4, respectively). Thus, the best condition for the production of lipase from *Geotrichum candidum* by SSF using 60 g of CB was: 10% (w/w) of N, 15% (w/w) of CO, 40% (w/w) of W, which resulted in 29.4 U g^{-1} after 48 h at 35°C without stirring. Evaluating the same condition about the productivity, the best result was 0.72 U g^{-1} h^{-1} at 24 h, 15% higher than that achieved within 48 h which indicates that the processing time could be reduced if the desired goal was to maximize productivity rather than the production.

The result presented here was comparable to those obtained from other studies using the same lipase activity measurement: *Penicillium simplicissimun* using castor residue (44.8 U g^{-1}), *Penicillium restrictum* using babassu oil residue (30.3 g U^{-1}), *Aspergillus niger* using cocoa meal (11.67 U g^{-1}) (Amorim et al., 2013; Godoy et al., 2009; Gombert et al., 1999). Regarding the substrate used, Damasco et al. (2008) obtained 62.7 U g^{-1} of lipase from *Aspergillus niger* using corn grounds as inductor in SSF. Khoramnia et al. (2014) also used FSS and *Geotrichum candidum* (like in our study) have evaluated the medium chain fatty acid content on the fermentation of coconut flakes and the optimum conditions were: 29% (w/v)

moisture and 10.14% (w/v) oil, after 9 days at 30°C, resulting in 76% of coconut oil hydrolysis.

CONCLUSION

This study brings an alternative application of SSF to produce lipase from *G. candidum*, since this microorganism has been extensively studied for lipase production, but mostly by SF. In this study, it was possible to achieve a maximum productivity of 0.72 U g^{-1} h^{-1} at 24 h (16% less activity than before optimization) and a maximum lipase activity of 29.4 U g^{-1} after 48 h (almost the double than before optimization). The corn bran was shown to be feasible for lipase production by SSF and the results obtained are comparable to data reported in the literature for the same microorganism.

REFERENCES

Amorim GM, dos Santos TC, Pacheco CSV, Barreto IMA, Freire DMG, Franco M. 2013. Fermentação de farelo de cacau por *Aspergillus niger* para obtenção de lipase. Est Tecnol Eng 8:24-27.

Asses N, Ayed L, Bouallagui H, Rejeb IB, Gargouri M, Hamdi M. 2009. Use of *Geotrichum candidum* for olive mill wastewater treatment in submerged and static culture. Biores Technol 100:2182-2188.

Brabcová J, Demianová Z, Vondrášeka J, Jágrd M, Zarevúcka M, Palomo JM. 2013. Highly selective purification of three lipases from *Geotrichum candidum* 4013 and their characterization and biotechnological applications. J Mol Catal B: Enz 98:62–72.

Canli O, Kurbanoglu EB. 2012. Application of low magnetic field on inulinase production by *Geotrichum candidum* under solid state fermentation using leek as substrate. Toxicol Indust Health 28:894-900

Damasco MCT, Passianoto MA, Freitas SCD, Freire DMG, Lago RCA, Couri S. 2008.Utilization of agroindustrial residues for lipase production by solid-state fermentation. Braz J Microbiol 39:676-681.

Gervais P, Molin P. 2013. The role of water in solid-state fermentation. Biochem Eng J 13:85–101.

Godoy MG, Gutarra MLE, Maciel FM, Feliz SP, Bevilaqua JV, Machado OLT, Freire DMG. 2009.Use of a low-cost methodology for biodetoxification of castor bean waste and lipase production. Enz Microb Technol 44:317-322.

Gombert AK, Pinto AL, Castilho LR, Freire DMG. 1999. Lipase production by *Penicillium restrictum* in solid-state fermentation using babassu oil cake as substrate. Proc Biochem 35:85-90.

Khoramnia A, Ebrahimpour A, Beh BK, Lai OM. 2014.*In Situ* Bioconversion of Coconut Oil via Coconut Solid State Fermentation by *Geotrichum candidum* ATCC 34614. Food Bioproc Technol 7:784-794.

Maldonado RR, Aguiar-Oliveira E, PozzaEL, Costa FAA, Maugeri Filho F, Rodrigues MI. 2014A. Production of lipase from *Geotrichum candidum* using corn steep liquor in different bioreactors. J Amer Oil Chem Soc 91:1999-2009.

Maldonado RR, Macedo GA, Rodrigues MI. 2014B. Lipase production using microorganisms from different agro-industrial by-products. Int J Appl Sci Technol 4(1):108-115.

Maldonado RR, Burkert JFM, Aguiar-Oliveira E, Durrant L, Mazutti MA, Maugeri Filho F, Rodrigues MI. 2014C. Elucidation of the effects of inoculum size and age on lipase production by *Geotrichum candidum*. Biotecnol Aplic 31(3):216-221.

Maldonado RR, Burket JFM, Mazutti MA, Maugeri F, Rodrigues MI. 2012. Evaluation of lipase production by *Geotrichum candidum* in shaken flasks and bench-scale stirred bioreactor using different impellers. Biocatal Agricult Biotechnol 1:147-151.

Okino-Delgado CH, Fleuri LF. 2014. Obtaining lipases from byproducts of orange juice processing. Food Chem 163:103–107.

Singh MK, Singh J, Kumar M, Thakur IS. 2014. Novel lipase from basidiomycetes *Schizophyllum commune* ISTL04, produced by solid state fermentation of *Leucaena leucocephala* seeds. J Mol Catal B: Enz 110:92-99.

Sun ZT, Liu C, Du JH. 2008.Optimisation of fermentation medium for the detoxification of free gossypol in cottonseed powder by *Geotrichum candidum* G07 in solid-state fermentation with response surface methodology. Annals Microbiol 58:683-690.