Synergismus scyentifica UTFPR
ISSN 2316-4689
https://periodicos.utfpr.edu.br/synscy

Production of the exopolysaccharide lasiodiplodan in a stirred-tank bioreactor

Francini Yumi Kagimura [1] Mário Antônio Alves da Cunha [2] Thaís Vanessa Theis [3]
Aneli de Melo Barbosa [4] Robert Dekker [5]

07 abr. 2015

Resumo – Polissacarídeos com propriedades biotecnológicas e atividades biológicas podem ser obtidos de plantas, algas, fungos filamentosos, leveduras e bactérias. Entre os polissacarídeos com atividades biológicas destacam-se as D-glucanas, as quais têm recebido grande atenção devido a seu potencial de atuação como imunoprotetor e como agente potencial para o tratamento de diferentes doenças (hipercolesterolemia, diabetes, problemas cardiovasculares, câncer). Recentes estudos demonstram a produção de β-D-glucana extracellular por fungos filamentosos em cultivo submerso. Neste contexto, o fungo ascomiceto *Lasiodiplodia theobromae* MMPI produz um exopolissacarídeo do tipo (1→6)-β-D-glucana denominado lasiodiplodana. O presente trabalho reporta a produção de lasiodiplodana pelo *L. theobromae* MMPI conduzida em biorreator de mistura através de fermentação submersa. O fungo foi cultivado em meio mínimo de sais minerais contendo glicose (20 g/L) como substrato limitante, pH inicial de pH 5.5, velocidade de agitação de 400 rpm, fluxo de ar de 0.8 vvm por 72 h a 28 °C. Produção máxima de lasiodiplodana (9.53 g/L) foi verificada em 72 h. Foi obtido rendimento de 0.58 g/g em lasiodiplodana, rendimento em biomassa de 0.23 g/g, produtividade volumétrica em lasiodiplodana de 0.13 g/L.h e taxa de consumo de substrato de 0.23 g/L.h. Os resultados obtidos demonstraram que o *L. theobromae* MMPI apresentou elevada capacidade de produção de lasiodiplodana em fermentação submersa conduzida em biorreator de tanque de mistura. As condições de cultivo contribuíram tanto para a produção de lasiodiplodana como biomassa micelial.

Palavras-chave: β-Glucana. polissacarídeo. biopolímeros. fermentação.

1. INTRODUCTION

The ascomyceteous fungus *Lasiodiplodia theobromae* belongs to the Botryosphaeriaceae family, which represents the asexual form of Botryosphaeria rhodina (MUNIZ et al., 2011). Although this fungus is a phytopathogen prevalent in tropical and subtropical regions of the world, it is also known as a producer of several bioactive natural products (LIMA et al., 2012; TSUKADA et al., 2010).

Lasiodiplodia theobromae has been grown on solid

1 fran_yk@hotmail.com, UTFPR Câmpus Pato Branco, Pato Branco, Brasil.
2UTFPR Câmpus Pato Branco, Pato Branco, Brasil.
3UTFPR Câmpus Pato Branco, Pato Branco, Brasil.
4UTFPR Câmpus Pato Branco, Pato Branco, Brasil.
5UTFPR Câmpus Pato Branco, Pato Branco, Brasil.

medium and presents different characteristics in relation to fungal colour presentation and growth rate. There is also some variation in substrate degradation, temperature tolerance, and metabolite production (PEREIRA et al., 2006). The colour of *L. theobromae* colonies varies from grey to black depending upon the substrate used in the culture medium, and the aerial hyphae on the mycelial surface is abundant.

Among the metabolites produced by species of Lasiodiplodia, emphasis has been on the polysaccharides, D-glucans (VASCONCELOS et al., 2008; CUNHA et al., 2012), which belong to a group of biomacromolecules with α- and β-anomeric configurations, constituting the α- and β-D-glucans. These carbohydrate biopolymers have attracted attention because of their commercial applications in the chemical and biopharmaceutical sectors, as well as their rheological properties and biological activities (KAGIMURA et al., 2015).

Pullulan (SPATAREANU et al., 2014) and dextran (BASHARI et al., 2013) constitute examples of α-D-glucans. Among the β-D-glucans are the polysaccharides present in the cell walls of filamentous fungi including yeasts, mushrooms, and cereals such as oat and barley (PETRAVIĆ-TOMINAC et al., 2010).

β-Glucans also can be produced exocellularly by microorganisms (e.g., xanthan, scleroglucan, lasiodiplodan, botryosphaeran) and can easily be recovered from the fermented medium by precipitation with alcohol, followed by dialysis against distilled water (KAGIMURA et al. 2015).

Microbial β-glucans consist of polymers of glucose residues linked by glucosidic bonds (mainly (1→3), and (1→6)), and can also present (1→6)-linked branches along the main (1→3)-glucan chain. These types of β-glucans have presented various biological activities. For example, fungal β-glucans have been described to be effective in treating diabetes, microbial infections, Alzheimer disease, AIDS (Acquired Immune Deficiency Syndrome), multiple sclerosis, cardiovascular diseases, and even cancer (NOVAK AND VETVICKA, 2008).

An exocellular (1→3;1→6)-β-D-glucan, called botryosphaeran, and produced by Botryosphaeria rhodina MAMB-05 exhibited hypoglycaemic and hypocholesterolaemic activities (Miranda-Nantes et al., 2011). Another exopolysaccharide of the (1→3), (1→6)-β-D-glucan type from *Panebacillus polymyxa* exhibited antiproliferative activity against sarcoma S 180 cells, as well as antioxidant property (JUNG et al., 2011).

Lasiodiplodia theobromae MMPI secretes into the culture liquid medium an exopolysaccharide (1 ⅋)-β-D-glucan called lasiodiplodan, that possessed antiproliferative activity against MCF-7 breast cancer cells (CUNHA et al., 2012) and antioxidant activity (GIESE el at., 2015). The objective of the work reported here was to evaluate the production of lasiodiplodan by *L. theobromae* MMPI in a stirred-tank bioreactor using glucose as a limiting substrate.

2. MATERIAL AND METHODS

2.1. Microorganism and inoculum preparation

The fungus *Lasiodiplodia theobromae* MMPI (SALDANHA et al., 2008) was maintained through periodic transfer at 4 °C on Sabouraud agar medium containing chloramphenicol (0.05 g/L). Sabouraud agar plates were inoculated by taking a loopful of hyphae scrapped off the mycelium from *L. theobromae* grown on agar slants, and then incubated at 28 °C for 96 hours. All the hyphae from mycelium colonized on the agar surface (one plate by flask) were transferred to a 250 mL Erlenmeyer flask containing 100 mL of VMSM and 5 g/L glucose. The flask was agitated at 150 rpm at 28 °C for 48 hours, and thereafter the pre-culture was aseptically homogenized for 30 seconds, and the mycelial homogenate centrifuged. The mycelium was recovered and re-suspended in sterilized distilled water, and used to make up a standard solution with absorbance between 0.4 to 0.5 at 400 nm according to Steluti et

al., (2004), and was used to inoculate the bioreactor.

2.2. Evaluation of lasiodiplodan production in a stirred-tank bioreactor (STR)

Submerged fermentation was conducted in a bench Biostat B (B. Braun International, Germany) Bioreactor using a 2-L fermentation vessel equipped with temperature control (Figure 1A). The working volume was 1.0 L, the air flux and the turbine agitation speed were set at 0.8 vvm and 400 rpm, respectively, and fermentation was operated for 72 hours at 28 °C. The nutrient medium consisted of VMSM and glucose (20 g/L) as sole carbon source. The initial pH was adjusted to 5.5 with HCl (1 mol/L) and the inoculum volume used was 100 mL (Cunha et al., 2012). Samples were collected from the sample port on the fermenter at 24-hour intervals to determine pH value, the EPS (lasiodiplodan) concentration, and residual glucose content. At 72 h the fermentation run was stopped, and the fermenter contents (Figure 1B) centrifuged (1500 x g/30 minutes) to recover the mycelial biomass. The supernatant containing the cell-free culture fluid was dialysed exhaustively against distilled water at 4 °C in dialysis tubing (≅12,000 Da, 1.3 inch width, MW 11,331, Sigma-Aldrich, USA), and the EPS precipitated with 3 vol. ethanol (PA), and left overnight at 4 °C (Figure 1C). The precipitate (lasiodiplodan) was recovered by filtration, and was solubilized in distilled water at 60 °C, and thereafter again dialyzed against distilled water over six days at 5 °C, with three changes of water per day. The dialysate was freeze-dried and stored at -20 °C (Figure 1D). The mycelial biomass obtained was repeatedly washed with hot water to remove any bound EPS, and the washings discarded. The biomass was then dried in oven at 60 °C until constant weight, and measured gravimetrically. Lasiodiplodan was quantified gravimetrically by drying in an oven at 60 °C. Residual glucose content in the fermented broth was determined by the DNS method (MILLER, 1959). pH was measured using a digital pH meter.

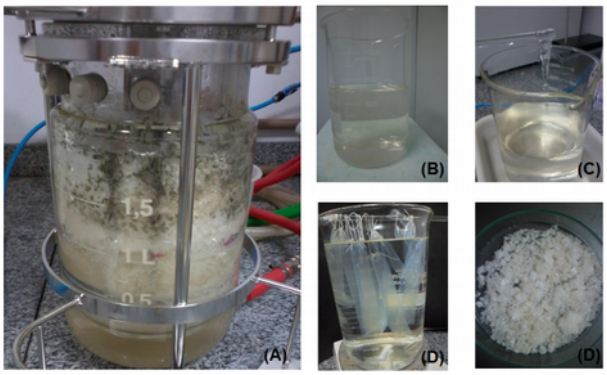

Figura 01 – Recovery process of lasiodiplodan: culture medium with *Lasiodiplodia theobromae* mycelium in the bioreactor (A), culture broth separated from the mycelial biomass (B), lasiodiplodan precipitated (C), dialysis process of lasiodiplodan (D), and freeze-dried lasiodiplodan (E).

Determination of fermentation parameters

In order to evaluate the fermentation profile of *L. theobromae* MMPI in the stirred-tank bioreactor, the following process parameters were determined: lasiodiplodan yield ($Y_{P/S}$), volumetric productivity of lasiodiplodan (Q_P), volumetric productivity of cell biomass (Q_X), substrate consumption rate (Q_S), percentage of substrate consumption (Y_C), biomass production per unit of glucose consumed ($Y_{X/S}$), and specific yield (Y_e).

The conversion yield of glucose into lasiodiplodan ($Y_{P/S}$) was calculated as the quantity of lasiodiplodan produced from the consumed substrate. The volumetric yield of lasiodiplodan (Q_p) was calculated as the relationship between the maximum lasiodiplodan concentration and the fermentation time. The volumetric yield of biomass (Q_x) was calculated as the relationship between the maximum biomass concentration and the fermentation time. The total substrate consumption rate (Q_S) was calculated as the relationship between the consumed glucose and the fermentation time. The percentage of substrate consumed (Yb) was calculated as the relationship between the consumed glucose and the initial glucose content. The biomass production per unit of glucose consumed (Yb) was determined as the relationship between the biomass produced and the glucose consumed. The specific yield (Y_e) was calculated as the quantity of lasiodiplodan produced per biomass formed.

2.3. Infra-Red-Fourier Transformation Spectroscopy (FT-IR)

FT-IR spectroscopy was conducted on lasiodiplodan using a FT-IR Spectrometer (Frontier, Perkin Elmer, USA) instrument in the wavelength region 4000-500 cm^{-1} using KBr discs.

2.4. Morphological analysis by Scanning Electron Microscopy (SEM)

The polymer surface of freeze-dried lasiodiplodan samples was analysed by SEM on an Electronic Scanning Microscope (Hitachi, model TM3000, USA). The sample was placed in the equipment support attached to carbon tape, and the scanned images were obtained at 800 and 2000 times magnification.

3. RESULTS AND DISCUSSION

3.1. Production of lasiodiplodan in a Stirred Tank Bioreactor

The operational and nutritional conditions used to cultivate *L. theobromae* MMPI contributed to cell growth, as well as to the production of lasiodiplodan (Figure 2). There was good mycelial growth and the fungus formed pellets similar to small spheres with diameters of ~2 mm. Some aerial mycelium grew at the interface of the culture medium, and also on the baffled wall of the bioreactor. Aerial mycelial growth (out of the broth culture) prevented an accurate estimation of the cell biomass during submerged fermentation, and so biomass was only determined at the end of the fermentation process.

Figura 02 – Submerged fermentation of *Lasiodiplodia theobromae* MMPI observed at 24 h (A), 48 h (B), and 72 h (C) in the stirred tank Bioreactor.

Mycelial growth over the fermentation process was prolific occurring mainly after 24 hours (observed visually) of cultivation. High cell biomass accumulated on the baffles and bioreactor wall at 48 hours (Figure 2B), and the amount of mycelial biomass produced was 3.87 g/L (dried weight) at the end of cultivation (72 hours).

Although the fungal isolate grew well under the culture conditions employed in this work, higher amounts of biomass was produced (11.9 g/L) in a previous study described by Cunha et al. (2012), when the same fungus was grown in a bench bioreactor. However, in that work, the amount of glucose used was twice (40 g/L) that used in this study, and the time of growth was longer (120 hours). In fact, higher amounts of substrate (glucose) can contribute to higher fungal growth as well as more EPS being produced. On the otherhand, higher concentrations of substrate associated with longer time of cultivation can contribute to stress in the microorganism, and the cell biomass darkens because of melanin production, which can also taint the EPS produced (DONG and YAO, 2012).

Figura 03 – Lyophilized lasiodiplodan.

In the present work, therefore, we opted to reduce the substrate concentration in the medium to 20 g/L, and shorten the cultivation time, in order to obtain the biopolymer without pigmentation attributable to melanin. Figure 3 shows the lasiodiplodan produced at the end of the fermentation run, which shows a

light colour, free of dark pigmentation.

The original pH of the culture medium was 5.5, which is considered ideal to grow *L. theobromae* MMPI (CUNHA et al. 2012). During the fermentation run, the pH fell from an initial value of 5.5 to 3.3 at 48 hours, and thereafter increased to 5.59 at 72 h. The pH decrease is probably associated with the production of organic acids arising from microbial metabolism (respiratory activity). The profile of EPS production and substrate utilization is shown in Figure 4. EPS is produced continuously during the first 48 hours, and then plateaux. At 48 hours the EPS concentration was 9.2 g/L, and at this stage only 78% of the glucose supplied to the fungus had been utilized. Although the cell biomass could not be quantified in 48 h because of the difficulties on quantitative sampling during the fermentation run, but at this stage the amount of biomass is probably very similar to the amount observed in the end of the fermentation process (72 h), as at this stage 81.35 % glucose had been consumed. According to Cunha et al. (2012), there is apparently a correlation between lasiodiplodan production and cell growth. As mentioned above, mycelial biomass could not be quantified at 24 and 48 h of cultivation because of mycelium adhering to the baffles and bioreactor wall, and this impeded the collection of representative samples of the cell biomass at the various sampling times during the fermentation run.

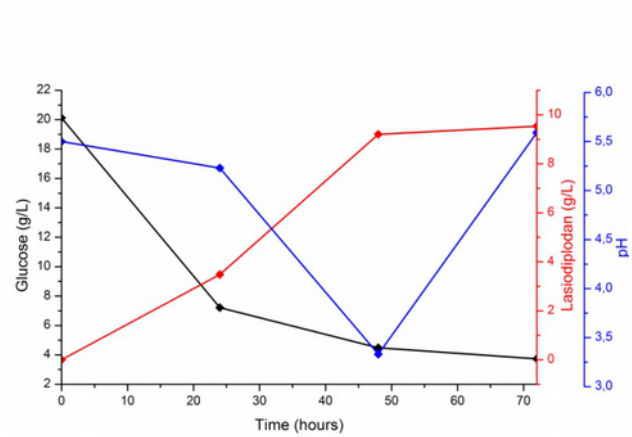

Figura 04 – Profile of glucose utilization, pH variation and lasiodiplodan production by L. thebromae MMPI over 72 h in the Stirred Tank Bioreactor.

From the profile of consumption of glucose (Figure 4), a linear glucose consumption rate (64%) was observed for the first 24 h of fungal growth with a total rate of substrate consumed of 0.54 g/L h (Qs), which was reduced to 0.07 g/L h between 24 and 72 h.

There appears to be a correlation between glucose consumption and the accumulation of lasiodiplodan in the fermented liquid culture, considering that after 48 h there was a reduction in the glucose consumption rate, and the amount of EPS produced was practically very small between 48 h (9.20 g/L) and 72 h (9.53 g/L). Cunha et al. (2012) described that after 48 h there was no further increase in lasiodiplodan production when *L. theobromae* MMPI was grown under similar culture conditions, but at a higher glucose concentration (40 g/L). An explanation for this effect may be due to a possible reduction in the amount of oxygen available for the fungal cells as a function of the increased viscosity in the liquid culture medium as the amount of EPS accumulated. The same condition was also verified in this work as the viscosity increased in the fermented broth during the fermentation process.

3.2. Fermentation parameters of lasiodiplodan production

The values of the fermentation parameters of submerged fermentation of *L. theobromae* MMPI during lasiodiplodan production are showed in Table 1.

L. theobromae MMPI consumed 81.4% of the initial glucose supplied in the culture medium, and the total rate of glucose consumed was 0.23 g/L, while the final lasiodiplodan production was 9.53 g/L. Glucose was not completely utilized at 72 h considering that 18.6% of the initial glucose content was still detected in the fermented broth. Cunha et al. (2012) obtained a percentage of substrate consumed (Y_C: 69.5%) after 120 h of culture. The incomplete substrate consumed can be associated to EPS accumulation in the culture medium, and consequent reduction of oxygen available to the cells what can hamper the fungal

metabolic activities.

Tabela 01 – Fermentation parameters of lasiodiplodan production by *L. theobromae* MMPI in submerged culture in a stirred tank Bioreactor during 72 h.

Fermentation Parameters	Observed Values
P_F (g/L)	9.53 ± 0.17
P_x (g/L)	3.82
$Y_{(P/S)}$ (g/g)	0.58 ± 0.01
$Y_{(X/S)}$ (g/g)	0.23 ± 0.01
Y_e (g/g)	2.50 ± 0.02
Y_c (%)	81.31 ± 0.91
Q_P (g/ L·h)	0.13 ± 0.002
Q_X (g/ L·h)	0.05
Q_S (g/ L·h)	0.23 ± 0.001

The yield values of lasiodiplodan and cell biomass were 0.58 g/g and 0.23 g/g, respectively. These values are interesting when compared with the data obtained by Cunha et al., (2012): 0.21 g/g of lasiodiplodan yield, and 0.43 g/g cell biomass conversion. Apparently, higher initial concentrations of substrate in the culture medium contribute to higher substrate conversion into biomass at 72 h of cultivation, but lower conversion into EPS.

Mahapatra and Banerjee (2013) described in their review that both the carbon source concentration and aeration are parameters of major importance for EPS production, and accordingly, EPS production by fungi is oxygen dependent. In this context, greater accumulation of cellular biomass in the medium associated with large amount of EPS could act as a barrier to oxygen transfer and assimilation by the microorganism. Such conditions of lower oxygen uptake by the cells can lead to reduced EPS biosynthesis.

3.3. Partial characterization of lasiodiplodan

FT-IR spectroscopy

The FT-IR spectrum of lasiodiplodan is shown in Figure 5, within the region between 4000 cm^{-1} and 500 cm^{-1}.

The strong absorption band at 3424 cm^{-1} is attributed to OH stretching. The peak at 2923 cm^{-1} is attributed to CH stretching of the CH2 groups, while the peak observed at 1648 cm^{-1} was attributed to the glucose ring (XU et al. 2009).

The bands observed in the region from 1350 cm^{-1} to 1450 cm^{-1} corresponded to symmetric deformation of CH2 and COH groups. Vibrations of symmetric stretching C-O-C (characteristic groups of sugars) appear around 1075 cm^{-1}, and the vibrations of asymmetric stretching are around 1269 cm^{-1} and 1247 cm^{-1}. The absorption in the region 890 cm^{-1} characterise β-configuration (WANG e ZHANG 2009).Lasiodiplodan was first characterised as a (1→6)-β-D-glucan type by Vasconcelos et al. (2008) employing methylation, FT-IR and 13C NMR analyses.

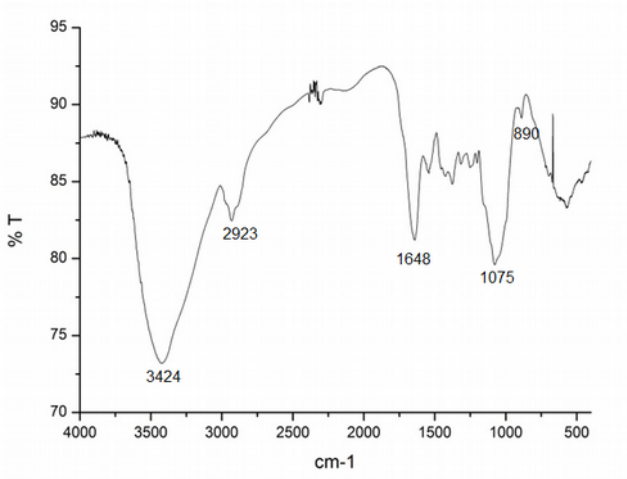

Figura 05 – FT-IR spectroscopy of lasiodiplodan.

Scanning electron microscopy (SEM)

Scanning electron micrographs of lasiodiplodan were obtained using a scanning electron microscope, Figure 6.

Figure 6 shows that the lyophilized EPS presents granules of ovoid shapes with average diameters of 3.33 μm (Figure 6B). The granules were distributed along an uneven surface similar to a dry and contorted "leaf".

A similar observation by SEM of the presence of ovoid shaped granules was reported by Jing et al. (2014) for a polysaccharide composed of glucose and rhamnose

produced by Cordyceps militaris. These authors verified structure composed by a surface in the form of "leaves" covered with (2 μm) ovoid shapes granules. According to these authors the structure similar to "leaves" is a morphology attributed to the polysaccharide network structure.

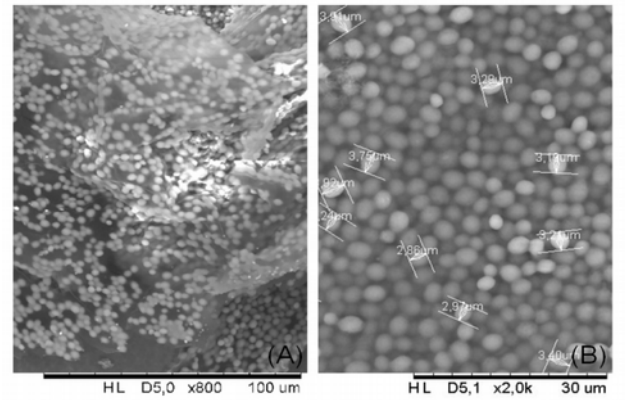

Figura 06 – Scanning electron micrograph of lasiodiplodan: structures in granule forms (A), granule diameters (B).

4. CONCLUSION

The fungus *L. theobromae* MMPI demonstrated good capacity to produce the EPS lasiodiplodan in submerged fermentation conducted in a Stirred-tank bioreactor. The operational conditions employed (20 g/L glucose, 400 rpm agitation using two Rushton turbine, 28 °C, 5.5 initial pH and 72 h cultivation time) contributed to good fungal growth as well as lasiodiplodan production. FT-IR spectroscopy showed typical signals of a $_{L}x$-D-glucan type, and the electron micrographs demonstrated the presence of spherical granules of oval shape, which were found distributed on the surface similar to contorted leaves.

Acknowledgments

The authors are grateful to CAPES, and Fundação Araucária do Paraná for the finance support..

Produção de exopolissacarídeo lasiodiplodana em biorreator de tanque

Abstract – Polysaccharides with biotechnical properties and biological activities can be obtained from plants, algae, fungi, yeasts and bacteria. Among the polysaccharides with biological activities are the D-glucans, which have received greatest attention because of their immunopotentiation, and as potential agents to treat human disease conditions (hypercholesterolemia, diabetes, multiple sclerosis, cardiovascular diseases, cancer). Recent studies have demonstrated the production of exocellular β-D-glucans by filamentous fungi in submerged cultivation. In this context, the ascomyceteous fungus, *Lasiodiplodia theobromae* MMPI, produces an exopolysaccharide of the (1→6)-β-D-glucan type, named lasiodiplodan. In the work reported here, the production of lasiodiplodan by *L. theobromae* MMPI by submerged fermentation was carried out in a stirred-tank bioreactor. The fungal isolate was cultured on minimum salts medium containing glucose (20 g/L) as limiting substrate, initial medium pH 5.5, impeller speed 400 rpm, airflow 0.8 vvm, for 72 h at 28 °C. Maximum production of lasiodiplodan (9.53 g/L) occurred at 72 h. The yield of lasiodiplodan was 0.58 g/g, biomass yield 0.23 g/g, volumetric productivity of lasiodiplodan 0.13 g/L.h and the rate of substrate consumption was 0.23 g/L.h. The results obtained indicate that the *L. theobromae* MMPI demonstrated hight lasiodiplodan production capacity in submerged fermentation using a stirrer-tank bioreactor. The culture conditions contributed both to the production of lasiodiplodan and mycelial biomass.

Keywords: β-Glucan. polysaccharide. biopolymers. fermentation.

REFERÊNCIAS

CUNHA, M. A. A.; TÚRMINA, J. A.; IVANOV, R. C.; BARROSO, R. R.; MARQUES, P.T.; FONSECA, E. A. I.; FORTES, Z. B.; DEKKER, R. F. H.; KHAPER, N.; BARBOSA, A. M. Lasiodiplodan, an exocellular (1→6)-β-D-glucan from *Lasiodiplodia theobromae* MMPI: production on glucose, fermentation kinetics, rheology and anti-proliferative activity. **Journal of Industrial Microbiology & Biotechnology**, v. 39, p. 1179–88, 2012.

DONG, C.; YAO, Y. Isolation, characterization of melanin derived from Ophiocordyceps sinensis, an entomogenous fungus endemic to

the Tibetan Plateau. **Journal of Bioscience and Bioengineering**, v. 113, p. 474–479, 2012.

GIESE, E. C.; GASCON, J.; ANZELMO, G.; BARBOSA, A. M.; CUNHA, M.A. A.; DEKKER, R. F. H. Free-radical scavenging properties and antioxidant activities of botryosphaeran and some other β-D-glucans. **International Journal of Biological Macromolecules**, v. 72, p. 125–130, 2015.

JING, Y.; CUI, X.; CHEN, Z.; HUANG, L.; SONG, L.; LIU, T.; LV, W.; YU, R.. Ellucidation and biological actibities of a new polysaccharide from cultured Cordyceps militaris. **Carbohydrate Polymers**, v. 102, p. 288–296, 2014.

LIMA, J. S.; CARDOSO, J. E.; MOREIRA, R. C.; ALVES, E. S.; MELO, J. G. M. Caracterização cultural de isolados de *Lasiodiplodia theobromae* e patogenicidade em plantas de aceroleira. **Revista Trópica - Ciências Agrárias e Biológicas**, v. 6, n. 1, p. 10–16, 2012.

MAHAPATRA, S.; BANERJEE, D.. Fungal Exopolysaccharide: Production, Composition and Applications. **Microbiology Insights**, v. 6, p. 3–16, 2013.

MUNIZ, C. R.; FREIRE, F. C. O.; VIANA, F. M. P.; CARDOSO, J. E.; COOKE, P.; WOOD, D.; GUEDES, M. I. F. Colonization of cashew plants by *Lasiodiplodia theobromae*: microscopical features. **Micron**, v. 42, n.5, p. 419–28, 2011.

NOVAK, M.; VETVICKA, V.. β-Glucans, History, and the Present: Immunomodylatory. **Aspects and Mechanisms of Action**, v. 5, p. 47–57, 2008.

PEREIRA, A. L.; SILVA, G. S.; RIBEIRO, V. Q. Caracterização Fisiológica, Cultural e Patogênica de Diferentes Isolados de *Lasiodiplodia theobromae*. **Fitopatologia Brasileira**, v. 31, n.6, 2006.

SALDANHA, R. L.; GARCIA, J. E.; DEKKER, R. F. H.; VILAS-BOAS, L. A.; BARBOSA, A. M. Genetic diversity among Botryosphaeria isolates and their correlation with cell wall-lytic enzyme production. **Brazilian Journal of Microbiology**, v. 38, p. 259–264, 2007.

STELUTI, R. M.; GIESE, E. C.; PIGGATO M. M.; SUMIYA, A. F. G.; COVIZZI, L. G.; JOB, A. E.; CARDOSO, M. S..; SILVA, M. L. C.; DEKKER, R. F. H.; BARBOSA, A. M. Comparison of Botryosphaeran production by the ascomyceteous fungus Botryosphaeria sp., grown on different carbohydrate carbon sources, and their partial structural features. **Journal of basic Microbiology**, v. 44, n. 6, p. 480–486, 2004.

TSUKADA, K.; TAKAHASHI, K.; NABETA, K.. Biosynthesis of jasmonic acid in plant pathogenic fungus, *Lasiodiplodia theobromae*. **Phytochemistry**, v. 71, p. 2019–2023, 2010.

VASCONCELOS, A. F. D.; MONTEIRO, N. K.; DEKKER, R. F. H.; BARBOSA, A. M.; CARBONERO, E. R.; SILVEIRA, J. L. M.; SASSAKI, G. L.; SILVA, R.; SILVA, M. L. C. Three exopolysaccharides of the β-(1→6)-D-glucan type and a β-(1→3;1→6)-D-glucan produced by strains of Botryosphaeria rhodina isolated from rotting tropical fruit. **Carbohydrate Research**, v. 343, n. 14, p. 2481–2485, 2008.

VOGEL, H. J. A convenient growth médium for Neurospora crassa. **Genetic Bulletin**, v. 13, p. 42–47, 1956.

WANG, J.; ZHANG, L. Structure and chain conformation of five water-soluble derivatives of a β-Dglucan isolated from Ganoderma lucidum. **Carbohydrate Research**, v. 344, p. 105–112, 2009.

XU, J.; LIU, W.; YAO, W.; PANG, X.; YIN, D.; GAO, X.. Carboxymethylation of a polysaccharide extracted from Ganoderma lucidum enhances its antioxidant activities in vitro. **Carbohydrate Polymers**, v. 78, p. 227–234, 2009.

Correspondência:
Francini Yumi Kagimura
fran_yk@hotmail.com, UTFPR Câmpus Pato Branco, Pato Branco, Brasil.
Recebido: 30/07/2014
Aprovado: 07/04/2015

Como citar (NBR 6023): KAGIMURA, Francini Yumi; CUNHA, Mário Antônio Alves da; THEIS, Thaís Vanessa; BARBOSA, Aneli de Melo; DEKKER, Robert. Production of the exopolysaccharide lasiodiplodan in a stirred-tank bioreactor. ***Syn. Scy.* UTFPR**, Pato Branco, v. 10, n. 1, p. 1–8, jan./mar. 2015. ISSN 2316-4689 (Eletrônico). Artigos convidados da SIMTEQ 2014, Pato Branco-PR. Disponível em: <https://periodicos.utfpr.edu.br/synscy>. Acesso em: DD mmm. AAAA.

DOI: "em processo de registro"