Anais da Academia Brasileira de Ciências (2018) 90(1): 461-470
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201820160333
www.scielo.br/aabc | www.fb.com/aabcjournal

Statistical tools application on dextranase production from *Pochonia chlamydosporia* (VC4) and its application on dextran removal from sugarcane juice

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Manuscript received on June 3, 2016; accepted for publication on August 15, 2016

ABSTRACT

The aim of this study was to optimize the dextranase production by fungus *Pochonia chlamydosporia* (VC4) and evaluate its activity in dextran reduction in sugarcane juice. The effects, over the *P. chlamydosporia* dextranase production, of different components from the culture medium were analyzed by Plackett-Burman design and central composite design. The response surface was utilized to determine the levels that, among the variables that influence dextranase production, provide higher production of these enzymes. The enzymatic effect on the removal of dextran present in sugarcane juice was also evaluated. It was observed that only $NaNO_3$ and pH showed significant effect ($p<0.05$) over dextranase production and was determined that the levels which provided higher enzyme production were, respectively, 5 g/L and 5.5. The dextranases produced by fungus *P. chlamydosporia* reduced by 75% the dextran content of the sugarcane juice once treated for 12 hours, when compared to the control treatment.

Key words: Sugarcane juice, dextranase, *Pochonia chlamydosporia*, Plackett-Burman, response surface.

INTRODUCTION

Dextran is the name given to a variety of extracellular polysaccharides composed of glucose units linked predominantly by α- (1,6) glycosidic linkages, but also containing α- (1,2) α- (1,3) and α- (1,4) (Morel du Boil and Wienese 2002). Its production is a common property to microorganisms in natural environments and its occurrence has been demonstrated in both microorganisms, prokaryotes and eukaryotes (Wingender et al. 1999).

Such substance is responsible for many problems in the sugar industry. Among them we highlight the reduction in the concentration and lower rate of sucrose recovery, increased sugarcane juice and syrups viscosity (Khalikova et al. 2005), and a quality deterioration of the finished product, sugar (Batista 2014, Morel du Boil and Wienese

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2002), besides the formation of opaque, irregular, elongated and caramel tone crystals (Jiménez 2009). Apart from the loss of sucrose and the final product quality deterioration, its presence in the sugarcane juice also results in increased equipment wear and compromise of the sucrose content quantification, which is used as the base for sugarcane producer's payment (Singleton et al. 2002).

Dextranases are enzymes that specifically break down dextran molecules of high molecular weight into smaller dextrans, thereby reducing the viscosity of both, massecuite and molasses. With dextranase use, the elongation of the grain ceases, the boiling time is shortened, and the product flows more smoothly due to reduced viscosity (Cuddihy et al. 2000, Jiao et al. 2014).

Dextranase production was verified in some species of bacteria, filamentous fungus and in a few yeasts and their use has taken place in several countries (Bhatia et al. 2010). Among the bacteria that produce dextranases, stand out those from the genus *Bacillus* (Esawy et al. 2012, Mahmoud et al. 2014, Zohra et al. 2013), *Bacteroides*, *Pseudomonas*, *Thermoanaerobacter* and *Streptococcus* (Khalikova et al. 2005). Among the fungus species that produce dextranases, stand out *Paecilomyces lilacinus* (Bhatia et al. 2010, 2016) and species of the genus *Penicillium* (Mahmoud et al. 2014, Shukla et al. 1989, Sugiura et al. 1973), *Chaetomium* (Hattori et al. 1981, Khalikova et al. 2005, Virgen-Ortíz et al. 2015) and *Aspergillus* (El-Shamy and Atalla 2014, El-Tanash et al. 2011).

The fungus *P. chlamydosporia*, used in this present study, is a facultative parasite of eggs and female nematodes found in the soil in various regions of the world, having been isolated directly from soil samples or eggs and nematode cysts (Lelis 2014, Podestá 2015). Although there are numerous studies in the literature using the fungus *P. chlamydosporia* toward their ovicidal and nematicidal activity, and proteases and chitinases production (Araújo et al. 2008, 2009, Braga et al. 2008, 2010, 2011, Frassy et al. 2010, Silva et al. 2010, Soares et al. 2015, Tobin et al. 2008) there are no reports as to their use for dextranases production.

Therefore, the objective of this study was to optimize dextranases production by fungus *P. chlamydosporia*, and its applicability in removing the dextran compound present in the sugarcane juice.

MATERIALS AND METHODS

FUNGUS CULTIVATION

The isolated fungus *P. chlamydosporia* VC4 originated from Brazilian soil, provided by the Parasitology Lab of the Veterinary Medicine Department, Universidade Federal de Viçosa, was maintained on a 2% (w/v) solid Potato Dextrose Agar medium (PDA 2% (w/v)) under refrigeration, at 4°C. Before inoculation in the liquid culture medium, the fungus was activated, and peaked to a new petri dish with PDA medium. The colonies were transferred in the form of discs and incubated for 10 days at 28°C.

OBTAINMENT OF THE ENZYMATIC EXTRACT

For the inoculation in liquid culture medium, were removed from edges of the petri dishes four equally sized disks. These disks were inoculated into 250 ml Erlenmeyer flasks containing 100 ml of liquid medium previously autoclaved at 121°C for 15 minutes. The liquid medium contained dextran 10 g/L, $NaNO_3$ 10 g/L, KH_2PO_4 4 g/L, $MgSO_4.H_2O$ 0.5 g/L, KCl 0.5 g/L, $ZnSO_4.7H_2O$ 0.178 g/L, $FeSO_4.7H_2O$ 0.18 g/L. After inoculation, the flasks were kept at 28°C and 180 rpm for 7 days. After 7 days, the Erlenmeyer flasks content was filtered and centrifuged at 10,000 X g for 20 minutes, yielding the crude extract.

ENZYME ACTIVITY MEASUREMENTS

The dextranase activity was measured by assessing the amount of reducing sugar produced from dextran hydrolysis by the 3,5-Dinitrosalicylic acid

method (DNS) (Miller 1959). The enzymatic assay was performed in test tubes, using 750 µL sodium acetate buffer 100 mM pH 5.2, 200 µL of dextran solution at 1% and 50 µL of the enzyme sample. The reactants were incubated at 50°C for 15 minutes. After this time, the reaction was stopped by adding 1 mL of the DNS reagent. Samples were heated for 5 minutes in a water bath. After the boiling bath, 2 mL of water was added to each test tube. The absorbance readings were performed at 540 nm. In order to obtain the amount of reducing sugar from the absorbance values, a standard curve with increasing concentration of glucose was constructed. One dextranase unit (U) was defined as the amount of the enzyme that catalyzes the release of 1 µmol of reducing sugar per minute under the assay conditions. All enzyme activity measurements were performed in triplicate.

INCUBATION TIME EFFECT ON DEXTRANASE PRODUCTION

During a time period of 11 days, every day, an aliquot of 1 mL was removed from each one of the three replicate flasks, from each treatment, transferred to a microtube and centrifuged at 10,000 X g for 20 minutes. These samples were measured for dextranase activity. The liquid culture medium was prepared as described before. Data were analyzed using the software Bioestat 5.0 (Belém, Pará, Brazil), by analysis of variance (ANOVA) with significance level of 5 and 1%. Subsequently, the data were analyzed by Tukey test, with 5% significance.

ENZYMATIC CHARACTERIZATION

pH and temperature effect on enzyme activity

In order to access the pH effect over the dextranase activity, an assay was conducted using 100 mM citrate-phosphate buffer with different pH values: 2.2; 3.0; 4.0; 5.0; 6.0; 7.0 and 8.0. Assays were performed under the conditions described above, using the buffer solution in such pH values.

The dextranase activity was measured using reactions with different incubation temperatures: 30, 40, 50 and 60°C, keeping all the previously described conditions, except for the buffer pH, where citrate-phosphate buffer solution 100 mM utilized had a pH value of 6.0.

Reaction time effect on enzyme activity

The dextranase activity was measured using different reaction incubation times: 5, 10, 15, 20, 25 and 30 minutes. In this assay, and all other following, were used the temperature and the pH which resulted in higher enzyme activity.

Ingel activity assay

In order to confirm the dextranase activity presented on the crude extract of the fungus *P. chlamydosporia,* an in-gel activity assay was warried out. The crude extract was applied to 10% native PAGE gel containing 1% blue dextran, and a voltage of 80 V was used. The gel was incubated in citrate phosphate buffer 100 mM pH 6.0 for 1 hour at 50°C. The dextranase activity was detected as a clear band on blue background.

EXPERIMENTAL DESIGN

In order to determine which culture medium components significantly influence dextranase production by the fungus *P. chlamydosporia* (VC4), it was evaluated the following variables by the Plackett-Bruman design (Plackett and Burman 1946): dextran (g/L), $NaNO_3$ (g/L), KH_2PO_4 (g/L), salts ($MgSO_4.H_2O$, KCl, $ZnSO_4.7H_2O$, $FeSO_4.7H_2O$) (g/L), pH and incubation time (days). For this analysis, Minitab 16 software (Philadelphia, Pennsylvania, USA) was used and each factor was analyzed at two levels: -1 to +1 and lower level to the upper level. The variables and their analyzed levels are shown in Table I. The experimental design matrix is illustrated in Table II.

TABLE I
Lower (-1) and higher (+1) level of the six variables analyzed by the Plackett-Burman design.

Variables		Lower level (-1)	Higher level (+1)
Dextran (g/L)		2	20
$NaNO_3$ (g/L)		0	10
KH_2PO_4 (g/L)		0	4
Salts (g/L)	$MgSO_4$	0	0.5
	KCl	0	0.5
	$ZnSO_4$	0	0.178
	$FeSO_4$	0	0.180
pH		4.5	7.5
Time (days)		7	11

A central composite design is the most commonly used response surface designed experiment. Central composite designs are a factorial or fractional factorial design with center points, augmented with a group of axial points that let estimate the curvature (Machado 2009). This design was used to apply the response surface methodology, and thus analyze the variables levels obtained from the Plackett-Burman design, as well as their possible interactions. Each variable had its effect evaluated into five experimental levels: -α, -1, 0, +1, + α, where $\alpha = 2^{n/4}$, being n the number of variables and 0 corresponding to the central point. A total of 13 experiments were made, 5 of them being replicates of the center point. Data were analyzed using the software Design Expert 7.0. The variables and their analyzed levels are shown in Table III. The experimental design matrix is shown in Table IV. The other variables not selected in the Plackett-Burman design were maintained as previously described.

DEXTRANASE APPLICATION IN THE DEXTRAN REMOVAL FROM THE SUGARCANE JUICE

For the sugarcane juice extraction, the sugarcane cultivar RB867515 used in this study was obtained from the Department of Animal Science at Universidade Federal de Viçosa. After the harvest, the sugarcane was stored at room temperature for 7 (seven) days. The sugarcane juice was extracted from 5 (five) sugarcanes through a grinder and, subsequently, filtered to remove all residues. The dextran quantification from the sugarcane juice proceeded immediately after extraction. Different amounts of dextranase (0, 5, 10 and 15 U) from the crude extract was added to 100 mL of sugarcane juice, and the residual dextran was quantified

TABLE II
Plackett-Burman design matrix and the results for dextranase activity.

Trial	Dextran (g/L)	$NaNO_3$ (g/L)	KH_2PO_4 (g/L)	Mineral Salts				pH	Incubation Time (dias)	Activity (U/mL)
				$MgSO_4$ (g/L)	KCl (g/L)	$ZnSO_4$ (g/L)	$FeSO_4$ (g/L)			
1	20	0	4	0	0	0	0	4.5	7	0.308
2	20	10	0	0.5	0.5	0.178	0.180	4.5	7	0.553
3	2	10	4	0	0	0	0	7.5	7	0.875
4	20	0	4	0.5	0.5	0.178	0.180	4.5	11	0.557
5	20	10	0	0.5	0.5	0.178	0.180	7.5	7	0.748
6	20	10	4	0	0	0	0	7.5	11	0.732
7	2	10	4	0.5	0.5	0.178	0.180	4.5	11	0.553
8	2	0	4	0.5	0.5	0.178	0.180	7.5	7	0.393
9	2	0	0	0.5	0.5	0.178	0.180	7.5	11	0.600
10	20	0	0	0	0	0	0	7.5	11	0.582
11	2	10	0	0	0	0	0	4.5	11	0.599
12	2	0	0	0	0	0	0	4.5	7	0.341

TABLE III
Levels of the two variables by the central composite design (CCD).

	Levels				
Variable	**-α**	**-1**	**0**	**+1**	**+α**
$NaNO_3$ (g/L)	1.89	5	12.5	20	23.11
pH	4.88	5.5	7	8.5	9.12

TABLE IV
Central composite experimental matrix design, using two variables and their respective dextranase activity values.

Trial	$NaNO_3$ (g/L)	pH	Dextranase Activity (U/mL)
1	5	5.5	4.35
2	20	5.5	3.53
3	5	8.5	0.72
4	20	8.5	1.87
5	1.89	7	1.84
6	23.11	7	1.14
7	12.5	4.88	4.39
8	12.5	9.12	0.77
9	12.5	7	1.37
10	12.5	7	0.79
11	12.5	7	1.40
12	12.5	7	1.26
13	12.5	7	0.90

after 12 h. Dextran quantification was performed according to the Roberts method (Roberts et al. 1983). The obtained data were compared by analysis of variance (ANOVA) and by the Tukey test, with 5% significance, using the Bioestat 5.0 software.

RESULTS AND DISCUSSION

INCUBATION TIME EFFECT ON DEXTRANASE PRODUCTION

The dextranase production was monitored for 11 days (Figure 1). The highest production in absolute values occurred on the eighth day of incubation.

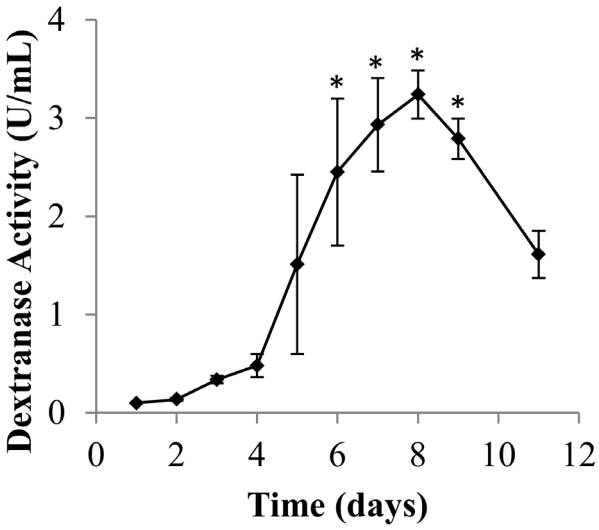

Figure 1 - Dextranase activity produced by the fungus *Pochonia chlamydosporia* as a function of the incubation time in submerged fermentation at 28°C and 180 rpm. *Indicates that there was no significant difference in the dextranase activity (p> 0.05) between the marked days by the Tukey test.

However, the dextranase activities on day 6, 7, 8 and 9 did not differed significantly (p>0.05). Therefore, for the other dextranase production tests, were used 7 days of incubation.

Bhatia et al. (2010) determined that *Paecilomyces lilacinus* reached maximum dextranase production at day 5 of incubation. When studying dextranase produced by *Fusarium moniliforme*, El-Masry (1991) noticed that most production occurred on day 8 of incubation, and Abdel-Naby et al. (1999) reported that *Penicillium funiculosum* had increased dextranase production on day 7 of incubation, which was also observed in this study.

ENZYMATIC CHARACTERIZATION

The enzyme reaction pH and temperature must be evaluated in order to determine the best conditions for the reaction to occur. It was evaluated the temperature (Figure 2) and pH (Figure 3) effect over dextranase activity. The dextranase activity was higher at 50°C and pH of 6.0, however the enzyme showed high activity between the pH ranging from 4.0 to 7.0. Given these results, the following trials

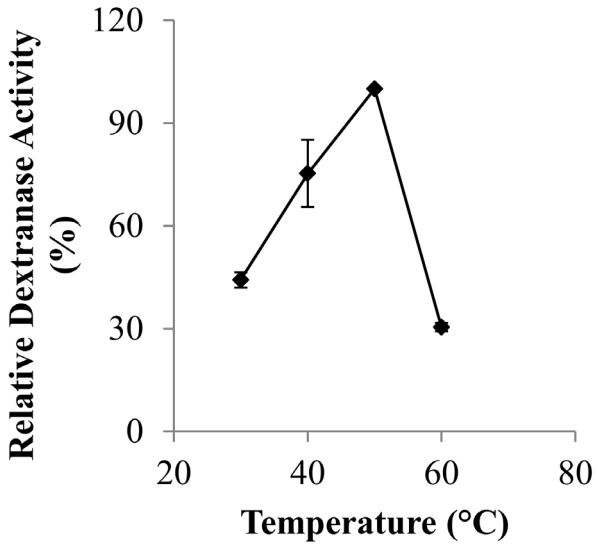

Figure 2 - Incubation temperature effect of the dextranase enzyme activity reaction present in the crude extract produced by the fungus *Pochonia chlamydosporia*.

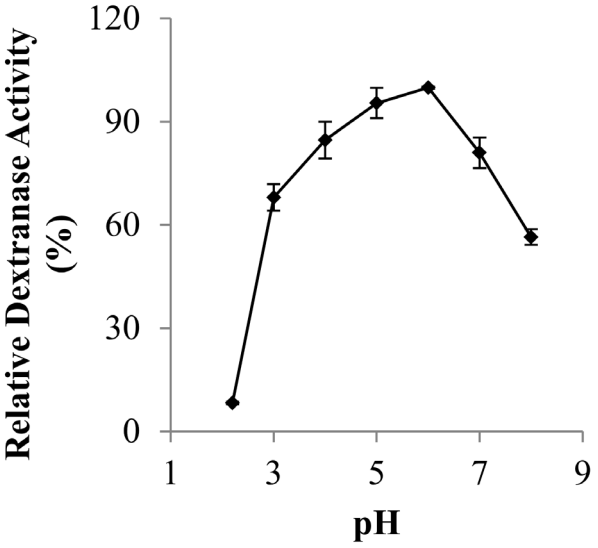

Figure 3 - pH effect of the dextranase enzyme activity reaction present in the crude extracts produced by the fungus *Pochonia chlamydosporia*.

on dextranase activity were conducted at 50°C and pH of 6.0.

These data corroborate with the results found by Bhatia et al. (2016), who determined that the dextranase produced by *P. lilacinus* was most active at 50°C and pH of 5. On the other hand, the

Figure 4 - Clear band showing the dextranase activity of crude extract on a 10% native PAGE gel containing 1% (w/v) blue dextran.

dextranase produced by *Aspergillus subolivaceus* showed higher activity at 60°C and pH of 5.5 (El-Tanash et al. 2011), and dextranase produced by *Penicillium lilacinum* exhibited maximum activity between the temperature and pH ranging from 30 to 35°C and 4.5 to 5.5, respectively (Aslan and Tanriseven 2007).

The in-gel activity assay demonstrates the existence of a band related to the dextranase activity, showing that there is only one form of dextranase present in the crude extract (Figure 4).

DEXTRANASE PRODUCTION OPTIMIZATION

The Plackett-Burman design allowed us to determine which, of the studied factors, significantly influence dextranase production from *P. chlamydosporia* fungus. It was evidenced that $NaNO_3$ significantly affect ($p<0.05$) dextranase production, and the pH showed a tendency to a significant effect ($p=0.054$) (Table V). The experimental matrix with dextranase activity values obtained from each run is shown in Table II.

TABLE V
Estimated effects of the variables studied in the Plackett-Burman design in the dextranase production by *Pochonia chlamydosporia*

	Effect	Coef	SE Coef	t-test	p-value
Intercept		0.5700	0.0337	16.88	0.000
Dextran	0.0199	0.0099	0.0337	0.30	0.780
$NaNO_3$	0.2129	0.1064	0.0337	3.15	0.025
KH_2PO_4	-0.0011	-0.0005	0.0337	-0.02	0.988
Salts	-0.0055	-0.0027	0.0337	-0.08	0.938
pH	0.1698	0.0849	0.0337	2.51	0.054
Time	0.0676	0.0338	0.0337	1.00	0.363

Coef: coeficient; SE: standard error; t-test: value of the variables determined by Student's t-test at 5% probability; p-value.

Based on the results from the Plackett-Burman experimental design, the central composite design was employed to establish the optimum levels of these two variables ($NaNO_3$ and pH). A total of 13 trials were conducted with different combinations of the two variables. The central point was repeated five times in order to estimate the error. The experimental matrix with the dextranase activity values obtained are shown in Table IV.

The regression model was statistically tested by the F test, and the analysis of variance (ANOVA) was used for the quadratic model of the response surface (Table VI). The quadratic model was significant with a p-value of 0.0003. The R^2 model was 0.94, indicating this model's reliability. The highest dextranase activity observed was 4.388 (U/mL) at pH of 4.88 and 12.5 (g/L) $NaNO_3$ concentration.

The results showed that the pH linear term, the pH quadratic term, and the term related to the interaction between pH and $NaNO_3$ have a significant effect ($p<0.05$) in the dextranase production from *P. chlamydosporia*.

The equation below presents the dextranase activity after the terms related to non-significant variables ($p>0.05$) have been removed:

$$Y=31.65860-6.92677X_1+0.047721X_1 X_2+0.38916X_1^2$$

Where Y is the dextranase activity, and X_1 and X_2 correspond to the pH and NaNO3, respectively.

As shown in Figure 5, a three dimensional response surface model was generated according to the final dextranase production from *P. chlamydosporia* fungus. It can be notice that the pH and $NaNO_3$ reduction results in a higher dextranase production. In other words, the dextranase activity was inversely proportional to the levels of variables pH and $NaNO_3$.

TABLE VI
Analysis of variance for the response equation obtained in the dextranase production by fungus *Pochonia chlamydosporia* in submerged fermentation.

Source	SS	DF	MS	F-Test	p-value
$NaNO_3$	0.088	1	0.088	0.48	0.5113
pH	14.00	1	14.00	76.21	<0.0001
$NaNO_3$*pH	1.15	1	1.15	6.27	0.0407
$NaNO_3^2$	0.77	1	0.77	4.16	0.0807
pH^2	5.33	1	5.33	29.02	0.0010
Model	20.91	5	4.18	22.76	0.0003
Lack of Adjustment	0.97	3	0.32	4.09	0.1036
Error	0.32	4	0.079		
Total	22.20	12			

SS: sum of the squares; DF: degree of freedom; MS: media square.

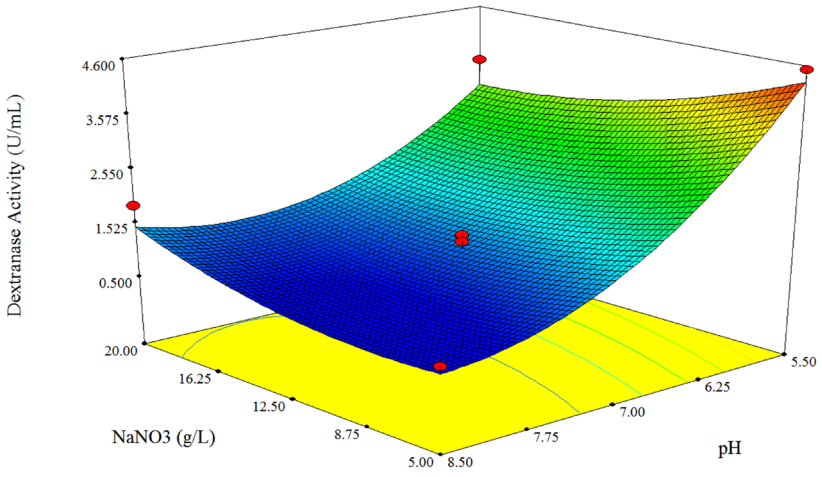

Figure 5 - Response surface of the dextranase production by the fungus *Pochonia chlamydosporia* in submerged fermentation.

DEXTRANASE APPLICATION IN THE DEXTRAN REMOVAL FROM THE SUGARCANE JUICE

The 12 hours sugarcane juice storage resulted in increased dextran content from 3.62 to 28.84 mg/100 mL. With the dextranase addition to the sugarcane juice, was observed a lower increase in the dextran content when compared to the control treatment, which demonstrates the dextran removal through the dextranase enzyme activity (Table VII). With the dextranase addition in a concentration of 15 U/100 mL, the dextran content measured after 12 hours was 7,158 mg/100 mL, which means that a reduction of 75% on the dextran content occurred when compared to the untreated treatment (28.84 mg/100 mL) (Table VII). Tukey's test revealed no significant difference ($p>0.05$) between the sugarcane juice treated with 10 and 15 U/100 mL of sugar cane juice. The dextran removal from the sugarcane juice through the use of dextranase produced from fungus was reported by Bhatia et al. (2016). In this particular study, when they added 5, 10 and 15 U of partially purified dextranase from *P. lilacinus* per 100 ml of sugarcane juice observed, after 12 hours, a reduction in the dextran content of 38.89%, 52.74% and 61.11%, respectively.

Among the analyzed factors, pH and $NaNO_3$ influenced the dextranase production of by fungus *P. chlamydosporia*. Through the surface response analysis, the $NaNO_3$ and pH levels that resulted in higher dextranase production were, respectively, 5.5 and 5g/L.

The dextranases produced by *P. chlamydosporia* proved to be effective in removing dextran present in sugarcane juice. After 12 hours, when comparing the untreated treatment with the

TABLE VII
Dextran removal in sugarcane juice by dextranase application at different concentrations.

Dextranase (unit/100 ml of sugarcane juice)	Dextran (mg/100 mL of sugarcane juice) after the time (h)	
	0	12
0	3.62 ± 0.10	28.84 ± 5.58 [a]
5	3.62 ± 0.10	16.10 ± 0.69 (44%) [b]
10	3.62 ± 0.10	10.06 ± 1.27 (65%) [bc]
15	3.62 ± 0.10	7.158 ± 3.76 (75%) [c]

The values shown are the mean ± standard deviation. The values in parentheses represents the percentage (%) of dextran content removal when compared to the control treatment (without dextranase). Values followed by the same letter were not significantly different ($p>0.05$) by Tukey test.

treated (15 U) treatment, there was a 75% dextran content reduction.

This is the first report on dextranase activity from the nematophagous fungus *P. chlamydosporia* and it opens new perspectives about the use of this established biological controller in a new biotechnological approach.

ACKNOWLEDGMENTS

The authors would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support.

REFERENCES

ABDEL-NABY MA, ISMAIL AS, ABDEL-FATTAH AM AND ABDEL-FATTAH AF. 1999. Preparation and some properties of immobilized *Penicillium funiculosum* 258 dextranase. Process Biochem 34: 391-398.

ARAÚJO JM, ARAÚJO JV, BRAGA FR, CARVALHO RO, SILVA AR AND CAMPOS AK. 2009. Interaction and ovicidal activity of nematophagous fungus *Pochonia chlamydosporia* on *Taenia saginata* eggs. Exp Parasitol 121: 338-341.

ARAÚJO JV, BRAGA FR, SILVA AR, ARAÚJO JM AND TAVELA AO. 2008. *In vitro* evaluation of the effect of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium sinense* and *Pochonia chlamydosporia* on *Ascaris suum* eggs. Parasitol Res 102: 787-790.

ASLAN Y AND TANRISEVEN A. 2007. Immobilization of *Penicilium lilacinum* dextranase to produce isomlatooligosaccharides from dextran. Biochem Eng J 34: 8-12.

BATISTA MCT. 2014. Produção de dextranases a partir de bagaço de malte: caracterização e avaliação do potencial de aplicaçao em indústria sucroalcooleira, 169 p. Dissertação de Doutorado - Universidade Federal do Paraná.

BHATIA S, BHAKRI G, ARORA M, BATTA SK AND UPPAL SK. 2016. Kinetic and thermodynamic properties of partially purified dextranase from *Paecilomyces lilacinus* and its application in dextran removal from cane juice. Sugar Tech 18: 204-213.

BHATIA S, BHAKRI G, ARORA M, UPPAL SK AND BATTA SK. 2010. Dextranase production from *Paecilomyces lilacinus* and its application for dextran removal from sugarcane juice. Sugar Tech 12: 133-138.

BRAGA FR, ARAÚJO JM, SILVA AR, ARAÚJO JV, CARVALHO RO, SOARES FEF, QUEIROZ JH AND GÊNIER HLA. 2011. Ovicidal action of a crude enzymatic extract of fungus *Pochonia chlamydosporia* against *Ancylostoma* sp eggs. Rev Soc Bras Med Tro 44: 116-118.

BRAGA FR, ARAÚJO JV, CAMPOS AK, ARAÚJO JM, CARVALHO RO, SILVA AR AND TAVELA AO. 2008. *In vitro* evaluation of the action of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium sinense* and *Pochonia chlamydosporia* on *Fasciola hepatica* eggs. World J Microb Biot 24: 1559-1564.

BRAGA FR, ARAÚJO JV, CARVALHO RO, SILVA AR, ARAÚJO JM, SOARES FEF, GENIÊR HLA, FERREIRA SR AND QUEIROZ JH. 2010. Ovicidal action of a crude enzymatic extract of the fungus *Pochonia chlamydosporia* against cyathostomin eggs. Vet Parasitol 172: 264-268.

CUDDIHY JA, RAUH J, MENDEZ F AND BERNHARD C. 2000. Dextranase in sugar production: factory experience. JASSCT 20: 95-102.

EL-MASRY HG. 1991. Optimization of dextranase synthesis by a locally isolated *Fusarium moniliforme* (12). Zbl Mikrobiol 146: 185-192.

EL-SHAMY AR AND ATALLA SMM. 2014. Immobilization of dextranase by *Aspergillus penicillioides* NRC 39 and its properties. Afr J Microbiol Res 8: 3893-3900.

EL-TANASH AB, EL-BAZ E AND SHERIEF AA. 2011. Properties of *Aspergillus subolivaceus* free and immobilized dextranase. Eur Food Res Technol 233: 735-742.

ESAWY MA, MANSOUR SH, AHMED EF, HASSANEIN NM AND EL-ENSHASY HA. 2012. Characterization of extracellular dextranase from a novel halophilic *Bacillus subtilis* NRC-B233b a mutagenic honey isolate under solid state fermentation. E-journal of Chemistry 9: 1494-1510.

FRASSY LN, BRAGA FR, SILVA AR, ARAÚJO JV, FERREIRA SR AND FREITAS LG. 2010. Destruction of *Toxocara canis* eggs by the nematophagous fungus *Pochonia chlamydosporia*. Rev Soc Bra Med Tro 43: 102-104.

HATTORI A, ISHIBASHI K AND MINATO S. 1981. The purification and characterization of the dextranase of *Chaetomium gracile*. Agr Biol Chem 45: 2409-2416.

JIAO Y, WANG S, LV M, JIAO B, LI W, FANG Y AND LIU S. 2014. Characterization of a marine-derived dextranase and its application to the prevention of dental caries. J Ind Microbiol Biot 41: 17-26.

JIMÉNEZ ER. 2009. Dextranase in sugar industry: A review. Sugar Tech 11: 124-134.

KHALIKOVA E, SUSI P AND KORPELA T. 2005. Microbial dextran-hydrolyzing enzymes: Fundamentals and applications. Microbiol Mol Biol R 69: 306-325.

LELIS RT. 2014. Controle biológico de Echinostoma paraensei pelo fungo nematófogo *Pochonia chlamydosporia*, 48

p. Dissertação de Mestrado - Universidade Federal de Viçosa. (Unpublished).

MACHADO FPP. 2009. Produção, purificação e caracterização bioquímica da dextranase de *Paecilomyces marquandii*, 91 p. Dissertação de Doutorado - Universidade Federal de Viçosa.

MAHMOUD KF, GIBRIEL AY, AMIN AA, NESSRIEN MN, YASSIEN NM AND EL BANNA HA. 2014. Microbial production and characterization of dextranase. Int J Curr Microbiol App Sci 3: 1095-1113.

MILLER GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing Sugar. Anal Chem 31: 426-428.

MOREL DU BOIL PG AND WIENESE S. 2002. Enzymic reduction of dextran in process-laboratory evaluation of dextranases. Proc S Afr Sug Technol Ass 76: 435-443.

PLACKETT RL AND BURMAN JP. 1946. The Design of Optimum Multifactorial Experiments. Biometrika 33: 305-325.

PODESTÁ GS. 2015. Interação entre *Pochonia chlamydosporia* e rizobactérias no controle de *Meloidogyne javanica*, 73 p. Dissertação de Doutorado - Universidade Federal de Viçosa.

ROBERTS EJ, CLARKE MA AND GODSHALL MA. 1983. The analysis of dextran in sugar production. Proc Int Soc Sugar Cane Technol, 18th Congress 3: 1374-1383.

SHUKLA GL, MADHU AND PRABHU KA. 1989. Study of some parameters for the production of dextranase by *Penicillium aculeatum*. Enzyme Microb Tech 11: 533-536.

SILVA AR, ARAÚJO JV, BRAGA FR, ALVES CDF AND FRASSY LN. 2010. *In vitro* ovicidal activity of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium thaumasium*, and *Pochonia chlamydosporia* on *Trichuris vulpis* eggs. Vet Parasitol 172: 76-79.

SINGLETON V, HORN J, BUCKE C AND ADLARD M. 2002. A new polarimetric method for the analysis of dextran and sucrose. J Am Soc Sugarcane Technol 22: 112-119.

SOARES FEF ET AL. 2015. Action of proteases of the nematophagous fungi *Pochonia chlamydosporia* on *Ascaris suum* eggs of collared peccary (*Pecari tajacu*). Afr J Microbiol Res 9: 1883-1886.

SUGIURA M, ITO A, OGISO T, KATO K AND ASANO H. 1973. Studies on dextranase: Purification of dextranase from *Penicillium funiculosum* and its enzymatic properties. Biochim Biophys Acta 309: 357-362.

TOBIN JD, HAYDOCK PPJ, HARE MC, WOODS SR AND CRUMP DH. 2008. Effect of the fungus *Pochonia chlamydosporia* and fosthiazate on the multiplication rate of potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) in potato crops grown under UK field conditions. Biol Control 46: 194-201.

VIRGEN-ORTÍZ JJ, IBARRA-JUNQUERA V, ESCALANTE-MINAKATA P, ORNELAS-PAZ JJ, OSUNA-CASTRO JA AND GONZÁLEZ-POTES A. 2015. Kinetics and thermodynamic of the purified dextranase from *Chaetomium erraticum*. J Mol Catal B-Enzym 122: 80-86.

WINGENDER RJ, NEU TR AND FLEMMING H. 1999. Microbial Extracellular Polymeric Substances: Characterization, Structure and Function. Springer, Berlin, v. 1, 258 p.

ZOHRA RR, AMAN A, ZOHRA RR, ANSARI A, GHANI M AND QADER SAU. 2013. Purification, characterization and end product analysis of dextran degrading endodextranase from *Bacillus licheniformis* KIBGE-IB25. Int J Biol Macromol 78: 243-248.