STABILITY CELLULASES OF *Bacillus* sp. SMIA-2 WITH DETERGENTS AND COMMERCIAL PROTEASES®

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***Abstract*—** Enzymatic stability is undoubtedly one of the most important factors in biotechnology. In this sense, the stability of cellulases present in crude extracts of submerged cultures of *Bacillus* sp. SMIA-2 in the presence of commercial detergents and proteases® was studied. Cellulases were resistant to digestion by proteases which is an interesting feature of these enzymes for application in detergent formulations.
Keywords— Stability, enzyme, *Bacillus* sp. SMIA-2, cellulases

1 INTRODUCTION

Cellulases are a class of enzymes whose demand for use in the formulation of laundry detergents has been growing a great deal. These enzymes act on the exposed glycosidic bonds of the cellulose, leading to the removal of fibrils that are the most exposed part of the tissue.

Bacillus sp. SMIA-2, a thermophilic bacterium isolated from soils of the Campos dos Goytacazes municipality, produced cellulases when cultivated in submerged cultures containing pure and complex natural sources of cellulose (Oliveira et al., 2013). Interestingly, the secreted cellulases were able to hydrolyse both the amorphous (soluble-carboxymethylcellulose) and crystalline (insoluble-avicel) forms of cellulose. The avicellase (which acts on the insoluble cellulose) had a better pH range for the 6-9 and 40-80 °C temperature. On the other hand, carboxymethylcellulose (which acts on soluble cellulose) showed a better pH and temperature range of 7-9 and 40-80 ºC, respectively.

Thus, the objective of this work is to study the stability of *Bacillus* sp. SMIA-2 with Omo® and Bem-te-vi® detergents and commercial proteases® due to their biodegradable properties, ideal for replacing products that harm the environment and cause wear on materials and instruments.

2 MATERIAL E METHODS

Microorganism: The bacterium *Bacillus* sp. SMIA-2, isolated by Souza and Martins (2001), from soil samples from the Campos dos Goytacazes region, Rio de Janeiro, Brazil was used in this work.

Culture medium: The culture medium for the production of the cellulases contained ($g.L^{-1}$ distilled water): peptone, 1.0; KCl, 0.3; K_2HPO_4, 0.87; $MgSO_4$, 0.5; NaCl, 10.0; and traces of metals ($CaCl_2$, $2.2x10^{-3}$, ZnO, $2.5x10^{-3}$, $FeCl_3 \cdot 6H_2O$, $2.7x10^{-2}$, $MnCl_2 \cdot 4H_2O$, $1.0x10^{-2}$, $CuCl_2 \cdot 2H_2O$, $8.5x10^{-4}$, $CoCl_2 .6H_2O$, $2.4x10^{-3}$, $NiCl_3.6H_2O$, $2.5x10^{-4}$, H_3BO_3, $3.0x10^{-4}$; Na_2MoO_4, $1.0x10^{-3}$). To this basal medium was added 0.8 % (m / v) corn steep liquor (Sigma Aldrich) and 0.8 % (w/v) sugarcane bagasse and 0.8 % (w/v) of flour of the passion fruit peel. The pH of the culture medium was adjusted to 7.5 with 1.0 M NaOH and sterilized by autoclaving at 121 °C to 1 atm for 15 minutes.

Culture conditions: The bacteria were streaked in Petri dishes containing TSY medium ($g.L^{-1}$ distilled water): tryptone 20; NaCl 10; yeast extract 10 and agar 20. The plates were incubated in a QUIMIS oven (model Q 315 D26) at 50 °C for 18 hours. After this period, 5 ml of the basal culture medium were transferred to the plates to resuspend the cells which, with the aid of a sterile pipette, were subsequently sucked out. These cells were inoculated into 250 mL. Erlenmeyer flasks containing 50 mL of the respective growth medium, incubated for an additional 18 hours at 50 °C in a Thermo Forma Orbital Shaker, Ohio, under 150 rpm shaking. This medium was called inoculum.

Enzyme Production: Samples containing the culture medium were centrifuged in a centrifuge (HERMLEZ 382K, Wehingen, Germany) at 4500 rpm for 30 minutes at 4 °C to obtain the cell-free supernatant, which was used for dosing the activity enzymatic activity.

Enzyme assays: Enzyme activity was determined by quantification of the release of reducing sugars from the hydrolysis of avicel (Avicelase) and carboxymethylcellulose substrates as described by Costa et al., 2017. A unit (U) of the enzyme was defined as 1 μmol of equivalent reducing sugar released by the substrate per minute under the above described test conditions using a standard glucose curve at concentrations of 0.014 to 0.3 mg / mL.

Stability of cellulases with commercial detergents: Commercial detergents Omo® and Bem-te-vi® were used in cellulase stability tests. They were diluted in Tris-HCl buffer (0.05M, pH 8.0) to a final concentration of 7mg / mL to simulate wash conditions. They were then heat treated at 100°C for 30 minutes to inactivate the enzymes that are part of their formulation. After cooling of this solution containing detergent, crude enzyme-containing extract was added (0.5 mL) and the flasks were incubated at room temperature and at defined time intervals (15, 30, 45, 60, 120 and 240 minutes) samples were taken to determine the residual activity, which was compared to the control performed in the absence of the detergent.

Stability of cellulases with commercial proteases: To study the effect of commercial proteases on cellulase activity, the crude extract was incubated at room temperature for 15, 30, 45, 60, 120 and 240 minutes in the presence of commercial protease diluted in distilled water at a concentration of 1: 10. Residual activity (%) was determined under the standard conditions previously described. A control (absence of commercial protease) was performed and considered to be 100 % activity. To analyze the data, the statistical program used was SAS - Statistical Analysis System (2003), version 9.3. The results obtained in each experiment were performed in triplicate and submitted to analysis of variance (ANOVA) to compare the means of the different treatments at the same time, as between the means of the same treatment between different times by the Tukey test at the level of 5 % significance.

3 RESULTS AND DISCUSSION

The avicellase was stable in the presence of Omo® and Bem-te-vi® detergents for up to 120 minutes at room temperature, where it maintained 97% and 100% respectively of its activity. When incubated in these same detergents for 240 minutes, a residual activity decrease was observed around 50 %.

TABLE 1
RELATIVE ACTIVITY OF AVICELLASE AND CARBOXYMETHYLCELLULASE PRESENT IN THE CRUDE EXTRACT OF *BACILLUS* SP. SMIA-2 IN THE PRESENCE OF OMO® AND BEM-TE-VI® DETERGENTS. (100% AVICELLASE ACTIVITY = 1.09 U / ML AND 100 % CMCASE ACTIVITY = 0.47 U / ML).

Time	Bem-te-vi®		Omo®	
(minute)	Avicelase (%)	CMCase (%)	Avicelase (%)	CMCase (%)
0	100,0 [a] ± 0,0012	100,0 [a] ± 0,0014	100,0 [a] ± 0,0012	100,0 [a] ± 0,0014
15	100,0 [a] ± 0,0019	104,0 [a] ± 0,0007	99,0 [a] ± 0,0030	100,0 [a] ± 0,0007
30	100,0 [a] ± 0,0031	98,0 [a] ± 0,0012	99,0 [a] ± 0,0026	108,0 [a] ± 0,0019
45	101,0 [a] ± 0,0031	106,0 [a] ± 0,0019	97,0 [a] ± 0,0031	111,0 [a] ± 0,0019
60	101,0 [a] ± 0,0031	102,0 [a] ± 0,0021	97,0 [a] ± 0,0026	111,0 [a] ± 0,0012
120	100,0 [a] ± 0,0019	98,0 [a] ± 0,0007	97,0 [a] ± 0,0038	98,0 [a] ± 0,0012
240	47,0 [b] ± 0,0018	51,0 [b] ± 0,0007	52,0 [b] ± 0,0014	51,0 [b] ± 0,0012

[1]Means with equal letters in the same column do not differ significantly from $p \leq 0.05$, according to the Tukey test.

Still according to the results presented in Table 1, the CMCase when incubated in the presence of Omo® and Bem-te-vi® detergents for up to 120 minutes showed an increase of its activity, around 11 % and 2 %. Already when incubated for 240 minutes showed a decrease of its residual activities around 50 %.

Cellulases produced by Bacillus cereus showed excellent stability in commercial detergents, maintaining about 80 % of their stability after 1 hour of incubation at 40 and 50 °C (Banik and Prakash, 2004). According to some authors, at temperatures higher than 40 °C, the enzyme requires the addition of stabilizing agents such as calcium, so that the enzyme remains stable.

TABLE 2
RELATIVE ACTIVITY OF AVICELLASE AND CARBOXYMETHYLCELLULASE PRESENT IN THE CRUDE EXTRACT OF *BACILLUS* SP. SMIA-2 IN THE PRESENCE OF PROTEASE COMERCIAL® (100 % AVICELLASE ACTIVITY = 1.11 U / ML AND 100 % CMCASE ACTIVITY = 0.46 U / ML).

Time (minute)	Avicelase (%)	CMCase (%)
0	100,0 [a] ± 0,0009	100,0 [a] ± 0,0009
15	100,0 [a] ± 0,0019	100,0 [a] ± 0,0029
30	100,0 [a] ± 0,0019	98,7 [a] ± 0,0007
45	100,0 [a] ± 0,0019	98,7 [a] ± 0,0007
60	100,0 [a] ± 0,0019	98,7 [a] ± 0,0007
120	100,0 [a] ± 0,0019	98,7 [a] ± 0,0007
240	66,6 [b] ± 0,0019	58,7 [b] ± 0,0007

[1]Means with equal letters in the same column do not differ significantly at $p \leq 0.05$, according to the Tukey test.

In relation to cellulase stability in the presence of commercial proteases, there was no significant difference ($p \leq 0.05$) in the activity of these enzymes when incubated in the presence of the protease for up to 120 minutes according to table 2, indicating that this enzyme was not able to degrade the cellulases produced by *Bacillus* sp. SMIA-2. Therefore, cellulases showed good stability when subjected to commercial protease action for up to 120 minutes.

Proteases are the major classes of enzymes used in the formulation of enzymatic detergents, followed by amylases. It occurs that proteases have the ability to degrade azocasein and other proteins, potentially causing a decrease in the activity of other enzymes, which may be present in the same formulation. Thus, for the cellulases secreted by *Bacillus* sp. SMIA-2 may be employed as detergent additives, they should be resistant to digestion by proteases.

According to Caparrós et al. (2012), cellulases of *Bacillus* species generally show good stability with proteases, which is interesting for applications in detergent formulations. The characteristics presented by the cellulases of

Bacillus sp. SMIA-2 show the potential for application of enzymes in industrial processes, such as additives in detergent formulations.

4 CONCLUSION

The cellulases secreted by *Bacillus* sp. SMIA-2 were stable in commercial Omo® and Bem-te-vi® detergents for up to 120 minutes. Regarding the stability in the presence of commercial proteases, the avicellase was stable, but the CMCase showed a small decrease in its activity when submitted to the action of this enzyme.

ACKNOWLEDGEMENTS

The team thank CAPES for the financial support, the partnership between UENF and IFS Campus Glória, the Research Group on Technology and Food Processing (TecPA) and the egress graduation of IFS Campus Glória Ramon Canuto Vieira Nascimento for the collaboration.

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