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Cellulase Production by *Streptomyces viridobrunneus* SCPE-09 Using Lignocellulosic Biomass as Inducer Substrate

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Abstract An actinomycete strain, isolated from a soil sample under a sugar cane plantation in Brazil and identified as *Streptomyces viridobrunneus* SCPE-09, was selected as a promising cellulolytic strain, and tested for its ability to produce cellulases from agro-industrial residues. Sugar cane bagasse or wheat bran was tested as carbon source, and corn steep liquor tested as nitrogen source. Different concentrations of carbon and nitrogen were tested using factorial design to identify optimal cellulose production. The results showed that media containing wheat bran 2.0% (*w*/*v*) and corn steep liquid 0.19% (*w*/*v*) lead to the highest production, 2.0 U mL^{-1} of CMCase, obtained on the fifth day of fermentation. The pH and temperature profile showed optimal activity at pH 4.9 and 50°C. As for thermostability, endoglucanases were most tolerant at 50°C, retaining more than 80% of maximal activity even after 2 h of incubation. Zymogram analyses using supernatant from growth under optimized conditions revealed the presence of two CMCase bands with apparent molecular masses of 37 and 119 kDa. The combination of pH tolerance and CMCase production from agro-industrial residues by *S. viridobrunneus* SCPE-09 offers promise for future bioethanol biotechnologies.

Keywords *Streptomyces viridobrunneus* · CMCase · Corn steep liquor · Sugar cane bagasse · Wheat bran

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Introduction

Actinomycetes are a Gram-positive filamentous group of bacteria abundantly found in soil and the most economically important and biotechnologically valuable prokaryotes. They are responsible for the production of about half of the discovered bioactive secondary metabolites, notably enzymes [1]. The *Streptomyces* are the most important Genus in the Actinobacterial group, and are able to produce and excrete a large variety of enzymes, including those involved in the degradation of cellulose, hemicellulose and lignin [2–4]. The microbiology of Brazilian tropical soils is largely unknown, offering excellent unexplored habitats for the bioprospection of new species and enzymes belonging to this very promising group [2, 3, 5, 6].

Cellulose is a linear polymer of 8,000–12,000 glucose units linked together by β-1,4-glycosidic bonds and is found as a major component of plant biomass, being naturally degraded by cellulolytic fungi and bacteria. Cellulose hydrolysis to glucose is performed via the synergic action of three cellulolytic enzymes: endo-β-D-glucanase (EC 3.2.1.4) exo-β-D-glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) [7].

Cellulose is principally used as the raw material for paper and textile industries.There is a growing interest in glucose derived from cellulose hydrolysis, as carbon source for other industrial products. Glucose derived from cellulose, via industrial fermentation, can be used to produce an array of fuels, principally “bioethanol”. Indeed, recently, the search for cellulolytic strains aiming at second-generation ethanol production has been the focus of several studies around the world [8, 9]. In this process lignocellulosic biomass such as agricultural residues, among others, provide a low cost feedstock for reducing sugars aiming at bioethanol production, through the action of lignocellulolytic enzymes, with economic and environmental advantages.

Also the utilization of agricultural by-products and other natural compounds as components of microbial growth media may represent a cost reduction in the production of important enzymes. Wheat bran is one of the most common agro-industrial residues used as raw material for several processes and products. Industrial wheat bran usually accounts for 14–19% of the grain and comprises the outer coverings, the aleurone layer and the remnants of the starchy endosperm. It consists mainly of starch, (glucurono) arabinoxylans, cellulose, β-glucan, protein, and lignin [10]. Also sugarcane bagasse is one of the largest cellulosic agro-industrial by-products, and contains, approximately, 50% cellulose and 25% hemicellulose and lignin [11]. Both substrates have already been tested as the main carbon source for the production of several enzymes including cellulases [8, 9, 12, 13]. In addition, corn steep liquor, a major by-product of the corn wet-milling industry, is also an inexpensive substrate available on a large scale, and has shown to replace yeast extract very efficiently as a rich source of nutrients such as organic nitrogen and vitamins in microbial media [2, 5].

Studies dealing with cellulase production by actinomycetes using low-cost residues are scarce in literature. Our group has isolated cellulolytic strains from Brazilian tropical soil [3, 14] and has obtained promising results [2, 4]. In this paper, we have investigated an actinomycete strain recently isolated from a Brazilian soil under a sugar cane plantation. An experimental design was performed to study endo-β-1,4-glucanase (carboxymethylcellulase, CMCase) production using steam-treated sugarcane bagasse or wheat bran as carbon source, and corn steep liquor as nitrogen sources. Culture supernatants presenting the highest enzyme activity were used to evaluate the effect of pH and temperature on CMCase activity and to perform zymogram analyses.

Materials and Methods

Microorganism

SCPE-09 was isolated from a soil sample under a sugar cane plantation in Brazil, using the dilution plate technique. Based on 16S rDNA sequencing the strain was classified as belonging to the *Streptomyces* genus, with 99.5% similarity with *Streptomyces viridobrunneus*, being identified as a strain of this species. Stock cultures were maintained on yeast extract–malt extract–agar plates [15] containing (g/L) yeast extract, 4.0; malt extract, 10.0; glucose, 4.0 and agar, 15.0. Spore suspensions were prepared according to Hopwood et al. [16] after cultivation (28°C/15 days) in this same medium. Spores were maintained in 20% (*v*/*v*) glycerol at −20°C.

Preliminary Tests for Cellulolytic Capacity

Cellulolytic activities were assayed first through qualitative tests by growth on solid medium containing CMC and using Congo red to reveal CMC-degrading zones [17] and also growth on cellulose–azure medium and observing the degradation and the liberation of azure dye [18].

Cellulase activity was measured after cultivation in 125 mL Erlenmeyer flasks containing 25 mL of mineral salts solution [19]; (in g/L, KH_2PO_4, 3.0; $(NH_4)_2SO_4$, 2.6; K_2HPO_4, 6.0; $MgSO_4.7H2O$, 0.2;NaCl, 2.0 and $CaCl_2$, 0.002) supplemented with a trace element solution [15]; (in g/L, $FeSO_4.7H_2O$, 1.1; $MnCl_2.4H_2O$, 7.9; $ZnSO_4.7H_2O$, 1.5 and $CuSO_4.5H_2O$, 6.4). Steam-pretreated sugarcane bagasse (SCB) or wheat bran (WB) 1% (*w*/*v*) was added as the main carbon source, and corn steep liquor (CSL) 1.2% (*w*/*v*) was used as the main nitrogen source. Steam-pretreatment consisted of a steam explosion, where, after impregnation of the lignocellulosic material in water, the system was submitted to high pressure (7–50 atm) at 160–190°C and then immediately alleviated [20]. This heat treatment aims to disrupt lignocelullose, facilitating cellulase access to its substrate. After being dried, the final material was weighed as an irregular mixture of fiber pieces varying from powder to 1 cm. The initial pH of the medium was adjusted to 7.0. Culture medium was inoculated with 25 μL of spore suspension (2.7×10^9 spores mL^{-1}), incubated at 28°C, and shaken (200 rpm) for 5 days. Samples were collected daily, by passing the supernatant solutions through a glass microfiber filter (Millipore) and supernatants were tested in cellulase activity assays. Results were presented as an average of three replicates.

CMCase Production Using Experimental Design

Optimization of the concentration of WB or SCB, as C source, and CLS as N source, was carried out by employing a response surface methodology having CMCase activity (U L^{-1}) as the independent variable and C source (SCB or WB) and N source (CSL) concentrations as the dependent variables. A 2^2 full factorial central composite rotational design (CCRD) was used in order to generate 11 run combinations as described in Table 1 [21]. This design is represented by a second-order polynomial regression model, Eq. (1), to generate contour plots:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}{X_1}^2 + b_{22}{X_2}^2 \quad (1)$$

where, Y is the predicted response (CMCase activity); X_1 and X_2 the coded forms of the input variables (WB or SCB and CSL, respectively); b_0 a constant; b_1 and b_2 the linear

Table 1 Values of independent variables (WB or SCB concentration X_1 and CSL concentration X_2, respectively) used in CCD, showing the values observed and predicted by the mathematical model for CMCase production by *S. viridobrunneus* SCPE-09

Run	Coded setting levels		Actual leves (% *w/v*)		CMCase activity (U mL^{-1}) WB		CMCase activity (U mL^{-1}) SCB	
	X_1	X_2	X_1	X_2	O	P	O	P
1	−1	−1	1.0	0.4	0.506	0.678	0.605	0.552
2	+1	−1	3.0	0.4	0.883	1.203	0.615	0.453
3	−1	+1	1.0	1.4	0.774	0.742	0.337	0.381
4	+1	+1	3.0	1.4	0.099	0.216	1.101	1.036
5	−1.41	0	0.59	0.9	0.595	0.212	0.546	0.528
6	+1.41	0	3.41	0.9	0.120	0.212	0.784	0.920
7	0	−1.41	2.0	0.19	2.004	1.539	0.238	0.341
8	0	+1.41	2.0	1.59	1.062	0.887	0.665	0.631
9	0	0	2.0	0.9	1.161	1.213	0.486	0.486
10	0	0	2.0	0.9	1.181	1.213	0.486	0.486
11	0	0	2.0	0.9	0.944	1.213	0.437	0.486

Results are the mean of two experiments

O observed, *P* predict

coefficients; b_{12} a cross-product coefficient; b_{11} and b_{22} the quadratic coefficients. The test factors were coded according to the following regression equation:

$$\mathrm{xi} = (\mathrm{Xi} - X0)/\Delta\mathrm{Xi} \tag{2}$$

where, *xi* is the coded value and *Xi* the actual value of the independent variable, *X0* the actual value at the center point and ΔXi is the step change value.

Analysis of variance (ANOVA) was used to estimate the statistical parameters. The significance of the regression coefficients was determined by Student's *t* test; the second-order model equation was determined by Fisher's test. The variance explained by the model is given by the multiple coefficient of determination, R^2. STATISTICA (version 7.0) software from StatSoft Inc. was used for regression and graphical analysis.

The same medium used in the preliminary tests, supplemented with different combination of SCB or WB as carbon source and CSL as nitrogen source, was used for the experimental design (Table 1). Conditions for the inoculation, incubation, and filtration of supernatant for further analysis for the 11 media proposed were also the same.

Based on the CCRD experiment, a validation was performed using, in triplicates, the conditions suggested by the model. For the combination of wheat bran and corn steep liquor 2.0% WB and 0.19% CSL were used. For sugarcane bagasse and corn steep liquor the values were 3.0% SCB and 1.4% CSL.

Enzymatic Assay

CMCase activity was assayed by measuring the release of reducing sugars in a reaction mixture of 1.0 mL of the crude extract and 1.0 mL of 2.0% (*w/v*) CMC solution in 50 mM sodium citrate buffer (pH 4.8) incubated at 50°C for 10 min. Reducing sugars were assayed by the dinitrosalicylic acid method [22]. One unit (U) of CMCase activity

corresponded to 1 μmol of glucose equivalent released per minute under the assay conditions [23].

Crude Enzyme Partial Characterization

Supernatant solutions from *S. viridobrunneus* SCPE-09 cultivated on the optimized medium (WB 2.0% and CSL 0.19%) was used as crude enzyme. The temperature profile for CMCase activity, assayed as described above, was determined by varying the incubation temperature between 20°C and 100°C at pH 4.8. In the same way, CMCase activity was determined in the pH range of 2.0–10.0, with the following buffers (50 mM) incubated at 50°C: glycine-HCl for pH 2.0–3.0, sodium citrate for pH 3.0–6.0, sodium phosphate for pH 6.0–8.0, Tris–HCl for pH 8.0–9.0, and glycine–NaOH for pH 9.0–10.0.

To study the CMCase thermal stability, the supernatant was pre-incubated at 40, 50 and 60°C for 0.5, 1, 2, 4, 6, 8, 16, 20, and 24 h (pH 4.8).

The influence of several metal ions on CMCase activity was evaluated performing the enzymatic assay at pH 5.0 and 50°C after addition of each ion (magnesium, calcium, manganese, zinc, copper, potassium, iron, barium, and sodium in the chloride form) at 10 mM final concentration [24]. The influence of ethylene diamine tetracetic acid (EDTA) was also tested at the same concentration.

These experiments were conducted in duplicate, and results expressed as average values. The residual activity was measured by usual enzyme activity determination procedures.

Zymograms

The culture supernatants from cells grown on the optimized medium were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, containing 10% (*w*/*v*) polyacrylamide [25] and CMC (Sigma) 0.2% (*w*/*v*). Electrophoresis was carried out at constant voltage (150 mV) at 4°C for 2 h. Samples containing 500 mU of activity were loaded. For CMCase activity detection the gels were incubated for 15 min at 50°C in 50 mM sodium citrate buffer (pH 5.0) and submerged in 0.1% (*w*/*v*) Congo red solution for 10 min [26]. The gel was washed with 1 mol L^{-1} NaCl until visualization of enzyme bands. For the determination of the molecular weight of the enzymes by electrophoresis, molecular weight standards ranging from 37.4 to 176.5 kDa (Electrophoresis Calibration Kit from Pharmacia, UK) were used, and the corresponding gel was stained using the silver staining method.

Results and Discussion

The qualitative tests (Congo red and cellulose-azure) performed for assessing *S. viridobrunneus* SCPE-09 cellulolytic abilities identified this strain as promising and worthy of more detailed study. When the strain was tested for CMCase production in submerged fermentation, using WB or SCB (1.0%) and CSL (1.2%), best production was 0.440 and 0.130 U mL^{-1}, respectively, after 2 days cultivation (Fig. 1). To enhance CMCase production, an experimental design was then proposed. Table 1 presents the observed and predicted results, obtained after 5 days cultivation. CMCase activity varied from 0.99 to 2.00 U mL^{-1} for WB and 0.238 to 1.10 U mL^{-1} for SCB. The best result was obtained on

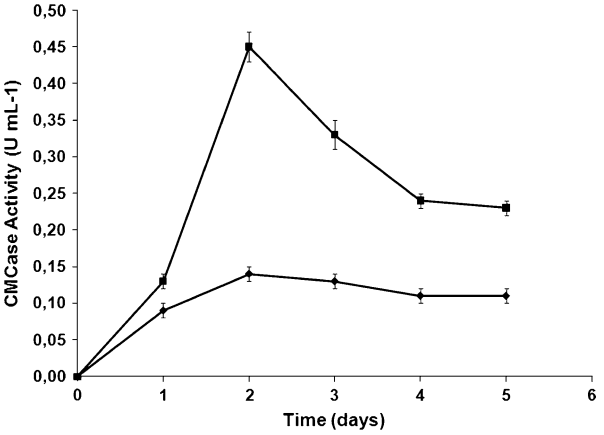

Fig. 1 Fermentation time course of CMCase production by *S. viridobrunneus* SCPE-09 on SCB (1.0% *w/v*; *filled diamond*) and WB (1.0% *w/v*; *filled square*) as carbon source, and CSL (1.2% *w/v*) as nitrogen source. *Error bars* represent standard deviation of each experimental point (n=3)

run number 7, where WB and CSL concentrations were 2.0% (*w/v*) and 0.19% (*w/v*), respectively. When SCB was the main carbon source the best result was obtained on run 4, where SCB and CSL concentrations were 3.0% (*w/v*) and 1.4% (*w/v*), respectively. Compared with the preliminary test (Fig. 1) an increase of 4.4-fold and 8.5-fold, for CMCase production using WB and SCB, respectively, was achieved using the factorial experimental design.

It was observed that the enzyme production levels are influenced by the carbon and nitrogen source concentrations. Generally best results of enzyme activity were obtained in the central point of C concentration for WB, and near the upper star point for SCB. As to the N source, low concentrations where better for WB, whereas high concentrations were better for SCB.

The model was tested for adequacy by the ANOVA. For WB+CSL combination (Table 2), the computed F value (8.44) indicates that the model was significant at a high confidence level. The probability P value was also very low (<0.1) indicating the significance of the model. The coefficient of variation (R^2=0.78) also indicates a very ood correlation between the experimentally observed and predicted values. The mathematical model representing the CMCase activity (Y) for the combination WB+CSL in the experimental region studied can be expressed by Eq. (3)

$$Y = 1,213.0 - 0.503{X_1}^2 - 0.231X_2 - 0.263X_1X_2 \quad (3)$$

Although the independent variable WB concentration had a significant effect on the CMCase production, interactions between WB and CSL had not (P>0.10).

For SCB+CSL combination (Table 3), the computed F value (10.64) indicates the significance of the model at a high confidence level. The probability P value was also very

Table 2 Statistical ANOVA for the model of CMCase production at different levels of concentration of WB and CSL

Source of variations	Sum of squares	Degrees of freedom	Mean square	F value	p value[a]
Regression	2.254	3	0.751	8.44	0.01
Residue	0.625	7	0.089		
Total SS	2.879	10			

Statistically significant at 90% of confidence level; R^2 =0.78

Table 3 Statistical ANOVA for the model of CMCase production at different levels of concentration of SCB and CSL

Source of variations	Sum of squares	Degrees of freedom	Mean square	*F* value	*p* value[a]
Regression	0.469	4	0.117	10.64	0.005
Residue	0.068	6	0.011		
Total SS	0.537	10			

[a] Statistically significant at 90% of confidence level; R^2 =0.87

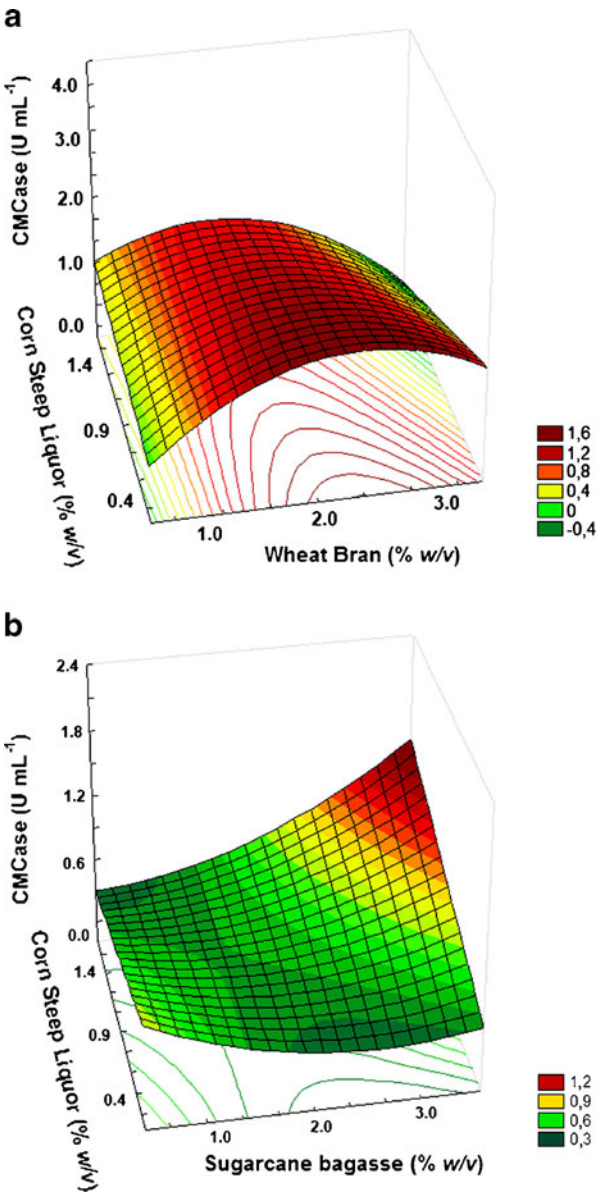

Fig. 2 Response surface on CMCase production by *S. viridobrunneus* SCPE-09 using WB and CSL (**a**) and SCB and CSL (**b**) concentration as dependent variables. The full factorial central composite design (2^2) used response surface methodology to predict the best point for CMCase production

low (<0.005) and the coefficient of variation (R^2=0.87) indicated again a very good correlation between the experimentally observed and predicted values. The independent variable SCB concentration had a significant effect on the CMCase production whereas interactions between WB and CSL had a high significance (P<0.10). The mathematical model representing the CMCase activity (Y) for the combination SCB+CSL can be expressed by Eq. (4).

$$Y = 0.486 + 0.139X_1 + 0.120{X_1}^2 + 0.103X_2 + 0.189X_1X_2 \quad (4)$$

The independent variable SCB concentration had also a significant effect on the interactions between WB and CSL

The regression analysis for the experiment using the combination WB+CSL and SCB + CSL, Eqs. 3 and 4, respectively, shows the significant coefficients of the full second-order polynomial model of CMCase production, determined by Student's t test and P values.

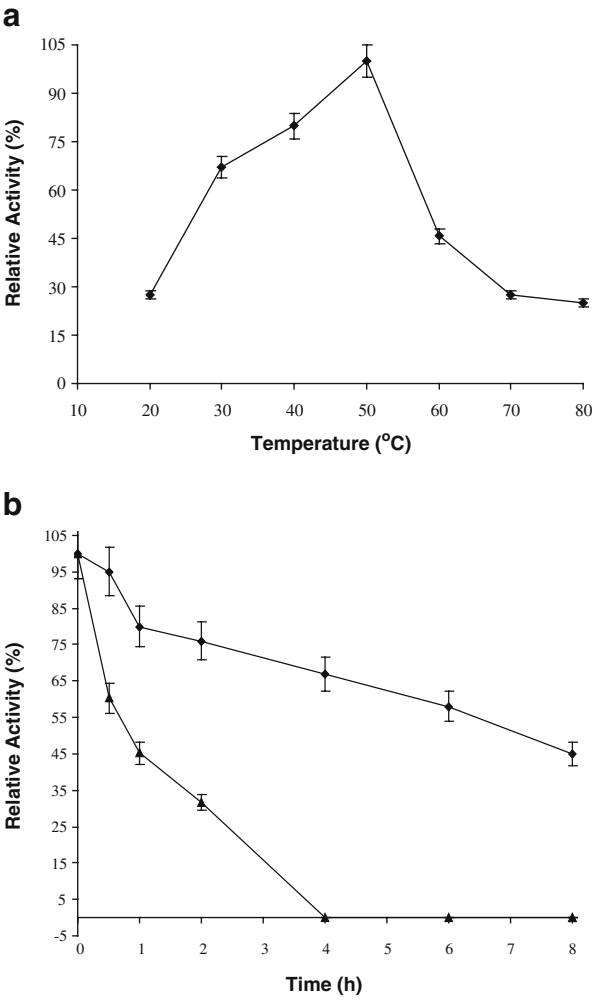

Fig. 3 Effect of temperature (**a**) and thermal stability (**b**) at 40 (*filled diamond*) or 50°C (*filled triangle*) on activity (pH 5.0) of cellulase produced by *S. viridobrunneus* SCPE-09 grown on 2.0% (*w*/*v*) WB and 0.19% (*w*/*v*) CSL. Residual activity is expressed as a percentage of the original activity. *Error bars* represent standard deviation of each experimental point (n=2)

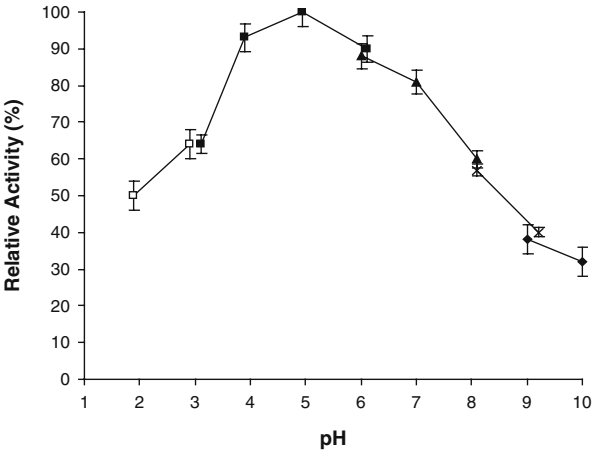

Fig. 4 Effect of pH on activity (50°C) of CMCase produced by *S. viridobrunneus* SCPE-09 grown on 2.0% (*w*/*v*) WB and 0.19% (*w*/*v*) CSL. The ionic strength for all buffers was 50 mM: glycine-HCl (*empty square*); sodium citrate (*filled square*); sodium phosphate (*filled triangle*); Tris–HCl (*multiplication symbol*); glycine-NaOH (*filled triangle*). Residual activity is expressed as a percentage of the original activity. *Error bars* represent standard deviation of each experimental point (n=2)

The resulting surface response plots showing the effect of substrate concentration (WB and CSL or SCB and CSL) on the CMCase production by *S. viridobrunneus* SCPE-09 are presented in Fig. 2a and b, respectively. Besides maximum CMCase activity, observed at 0.19% CSL and 2.0% WB, the surface response suggests another CMCase activity produced when a higher CSL concentration (1.6%) is used, as confirmed by the zymogram analysis (Fig. 5).

The validation of the mathematical model used based in CCDR experiments confirmed the maximal values for CMCase obtained, from 1.88 to 2.01 U/mL when using 2.0% WB+ 0.19% CSL and from 0.99 to 1.11 U/mL for 3.0% SCB+1.4% CSL.

According to literature, it is well known that actinomycetes, especially *Streptomycetes*, are able to degrade agro-industrial residues through lignocelluloytic enzymes, including cellulases. However, these studies were concerned with issues other than enzyme production. Few articles have been published describing cellulase production by actinomycetes using agro-industrial residues as substrates. Tuncer et al. [27] studied the production of endoglucanase, among other enzymes, with *Streptomyces* sp. F262 grown with 1.2% ball-milled wheat straw+yeast extract. Endoglucanase production was very low. Earlier, Godden et al. [28] reported that *Streptomyces* sp. EC1, grown on 0.2% ball-milled

Table 4 Effect of different ions on CMCase activity. Enzyme was produced by *S. viridobrunneus* SCPE-09 grown on 2.0% (*w*/*v*) WB and 0.19% (*w*/*v*) CSL

Ion[a]	Relative activity (%)[b]
Control (no addition)	100.0
EDTA	95.9
Mg^{2+}	86.4
Zn^{2+}	90.3
Cu^{2+}	13.1
Na^{+}	101.1
Ba^{2+}	91.4
Mn^{2+}	32.2
Ca^{2+}	98.8
K^{+}	65.4
Fe^{2+}	19.1

[a] The final concentration in the reaction mixture was 10 mM

[b] Relative activity is expressed as a percentage of control (100% of enzyme activity=2.00 U mL^{-1})

wheat straw in mineral salts supplemented with vitamins, resulted in 0.2 U mL^{-1} endoglucanase. Our group has been cultivating *Streptomyces* strains using agro-industrial residues aiming at production of lignocellulose degradation enzymes, including endoglucanases. The CMCase titres obtained with WB (2.00 U mL^{-1}) and SCB (1.10 U mL^{-1}) as carbon source and CSL as nitrogen source, under submerged culture conditions, obtained in this study were higher than the CMCase titers of 0.22 U mL^{-1} obtained by Grigorevski-Lima et al. [4] using WB and CSL, and 0.71 U mL^{-1} produced by *Streptomyces malaysiensis* on brewer's spent grain and CSL [2].

The temperature profile of CMCase activity present in the crude extract from *S. viridobrunneus* SCPE-09 grown in WB and CSL is presented in Fig. 3a. Maximal activity was observed at 50°C and activity values of approximately 80% and 45% were still detected at 40° and 60°C, respectively. These results are similar to or even somewhat higher than those reported by some authors for *Streptomyces* strains [29–31]. In our previous studies, we observed that the CMCase produced by *S. malaysiensis* was thermophilic, with residual activity at around 100% at temperatures between 40° and 60°C [2].

Thermostability experiments are shown on Fig. 3b. Crude enzyme was able to retain 76% residual activity at 50°C for 2 h, and 67% after 4-h incubation, the half-life of crude enzyme being 8 h at 50°C and 1 h at 60°C (data not show). Half-lives of 24 h at 40°C or 8 h at 50°C have been cited in literature for some *Streptomyces* strains [2, 4]. Our results strongly suggest that the CMCases in this supernatant are thermo tolerant and as such could be considered appropriate for many biotechnological processes.

The pH profiles (Fig. 4) demonstrate that 60% CMCase activity is maintained over a wide pH range (3.0–8.0), with optimal activity occurring at pH 5.0. This is a very peculiar and interesting characteristic, not very commonly described. There are very few reports in literature about CMCase activity over a very wide pH range. Most reports cite activity in the alkaline range only [14, 29, 31, 32]. Nascimento et al. [2] have shown a pH activity

Fig. 5 Zymogram analysis of CMCase activity in the supernatant of *S. viridobrunneus* SCPE-09 cultures grown on best condition. The amounts loaded in the gel contained 500 mU of cellulase activity. The gel containing the MW markers was stained for proteins using the silver staining method and values for the hydrolytic zones are shown on the left side of the figure

profile within the range 2.0–9.0, with maximum activity observed at pH 4.0. CMCase activity in the acid / alkaline pH range was also detected by Grigorevski-Lima et al. [4].

Results concerning CMCase activity in the presence of metal ions are shown in Table 4. Ions including Mg^{2+}, Zn^{2+}, Ba^{2+}, K^{+}, and also EDTA, Ca^{2+} and Na^{+}, had little or no significant effect on CMCase activity. In contrast, a considerable decrease in activity was observed in the presence of Cu^{2+}, Fe^{2+}, and Mn^{2+}. These ions are commonly cited in literature as inhibitors for several microbial cellulases [33–35], including *Streptomyces drozdowczii*, which was inhibited by Cu^{2+} [4]. Activity is probably inhibited through the attack of certain groups at the active site of the enzyme, for example, the thiol groups, leading to inactivation [33]. According to these results, these ions must be avoided in future cultivations for a good cellulase production.

The production of multiple cellulases is common in nature, especially in actinomycetes. Zymogram analyses of culture supernatants from *S. viridobrunneus* SCPE-09 confirmed this trait when the strain was grown in 2.0% WB (*w*/*v*) and 0.19% CSL (*w*/*v*) after 5-day fermentation (Fig. 5). In these conditions, two bands of CMCase activity were observed with estimated molecular masses of 37 and 119 kDa, respectively. Similarily, Nascimento et al. [2] observed three cellulolytic bands (51, 115, and 178 kDa) produced by *S. malaysiensis* using brewer's spent grain 0.5% (*w*/*v*) and corn steep liquor 1.2% (*w*/*v*).

Conclusion

The microorganism *S. viridobrunneus* SCPE-09 used in this study was able to grow and to produce CMCase using wheat bran or sugar cane bagasse and corn steep liquor as sources of C and N. The maximum CMCase activity detected was of 2.00 U mL^{-1}, on the fifth day of cultivation, when a salt mineral medium was supplemented with corn steep liquor 0.19% (*w*/*v*) and wheat bran 2.0% (*w*/*v*). These results were obtained after using a factorial experimental design for optimization. The experimental design resulted in a 2.8-fold improvement on CMCase production when compared with preliminary tests without factorial planning. The optimum of pH and temperature of crude extract were of 5.0 and 50°C, respectively, and the CMCase retained 100% of activity after 1 h at 45°C. Considering the low cost of the medium, and the high titers obtained for enzymatic activity, the results obtained indicate a possible use for these enzymes in biotechnology processes, especially for bioethanol production.

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