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Cellulase production by thermophilic *Bacillus* sp. SMIA-2 and its detergent compatibility

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ABSTRACT

Background: This paper reports the production of cellulase by thermophilic *Bacillus* sp. SMIA-2 using sugarcane bagasse and corn steep liquor as substrates. Some biochemical properties of the enzyme were also assessed for the purposes of exploiting its potential in the detergent industry, as well as other suitable applications.
Results: *Bacillus* sp. produced cellulases when cultivated at 50°C in liquid cultures containing sugarcane bagasse and corn steep liquor. Maximum avicelase (0.83 U mL^{-1}) and CMCase (0.29 U mL^{-1}) activities were reached in 120 h and 168 h of culturing time, respectively. The avicelase and CMCase presented an optimum activity at pH of 7.5 and 8.0, respectively. The maximum stability of avicelase and CMCase was observed at a pH range between 6.5–8.0 and 7.0–9.0 respectively, where they retained more than 70% of their maximum activities after incubation at room temperature for 3 h. The optimum temperature of avicelase and CMCase was 70°C, and both enzymes remained 100% stable until the treatment at 60°C for 1 h. *Bacillus* sp. cultures also released proteases into the culture medium, but the cellulases were resistant to protease digestion. The compatibility of cellulases varied with each laundry detergent tested, being more stable in the presence of Ultra Biz® and less with Ariel®. In addition, the enzyme was stable in sodium dodecyl sulfate and RENEX-95, and was inhibited by TritonX-100 and H_2O_2.
Conclusions: The properties presented by *Bacillus* sp. SMIA-2 suggest that this organism might become a potential source of lignocellulose-degrading enzymes for industrial applications such as in the detergent industry.

1. Introduction

Cellulase is one of the most useful enzymes in industry [1]. It is produced by fungi, bacteria or actinomycetes. Most commercial cellulases are of fungal origin; however, bacteria have also been considered as robust and versatile enzyme producers because of their high growth rate, stability at extreme conditions and, presence of multi enzyme complexes. Among bacteria, *Bacillus* species can produce several extracellular polysaccharidehydrolyzing enzymes, which include cellulase [2]. However, the study of *Bacillus* cellulase has, until recently, lagged behind that of fungal enzymes. This is largely due to the fact that most *Bacillus* cellulases hydrolyze synthetic carboxymethyl cellulose, but barely hydrolyze the crystalline form of cellulose [3,4,5].

The potential of cellulases has been revealed in various industrial processes, as cotton softening and denim finishing in the textile industry, in de-inking, in drainage improvement and fiber modification in the pulp and paper industries, as well as in laundry detergents as softening, anti-pilling, and color-reviving agents [6,7,8,9]. More specifically in laundry applications, fungal cellulases usually hydrolyze crystalline cellulose, though they are acidic and neutral enzymes, and show weak or no ability to hydrolyze crystalline cellulose in the alkaline region.

Cellulase has also been biosynthesized from lignocellulosic materials such as sugarcane bagasse, a fibrous residue of cane stalks left over after the crushing and extraction of the sugar from sugarcane. It is abundantly and cheaply available as a byproduct from the sugar industry in Brazil. Considering that the majority of strains of *Bacillus* sp. are often capable of utilizing the organic matter consisting of complex mixtures typical of most wastes, this work investigated the production of cellulase by *Bacillus* sp. SMIA-2 using sugarcane bagasse and corn steep liquor as substrates. Some biochemical properties of the enzyme were also assessed for the purposes of exploiting its potential in the detergent industry, as well as other suitable applications.

2. Materials and methods

2.1. Organism

The bacterial strain was a thermophilic *Bacillus* sp. strain SMIA-2, previously isolated from a soil sample collected in Campos dos

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Goytacazes city, Rio de Janeiro, Brazil. Phylogenetic analysis revealed that the bacterium was closely related to *Bacillus caldoxylolyticus* and *Bacillus* sp. strain AK1, and these three organisms exhibited levels of ribosomal DNA sequence homology of 94% [10].

2.2. Enzyme production

The culture medium for cellulase production contained (g/L): KCl—0.3, MgSO$_4$—0.5, K$_2$HPO$_4$—0.87, CaCl$_2$—0.29, ZnO—2.03×10^{-3}, FeCl$_3$·6H$_2$O —2.7×10^{-2}, MnCl$_2$·4H$_2$O—1.0×10^{-2}, CuCl$_2$·2H$_2$O—8.5×10^{-4}, CoCl$_2$·6H$_2$O —2.4×10^{-3}, NiCl$_3$·6H$_2$O—2.5×10^{-4}, H$_3$BO$_3$—3.0×10^{-4}, commercial corn steep liquor (Sigma Aldrich)—5.0 and sugarcane bagasse in natura—5.0.

Sugarcane bagasse was collected from a local sugar mill. It was prepared by exhaustive washing with distilled water to remove all residual sugars, dried at 70°C for 48 h, and ground in a blender to a fine powder. The average composition of this feedstock was 4.35% moisture, 42.43% cellulose, 28.96% hemicellulose, 18.61% insoluble lignin, and 1.47% ash.

The pH was adjusted to 7.2 with 1.0 M NaOH and the medium was sterilized by steam-autoclaving at 121°C, 1 atm for 15 min. The medium (50 mL in 250 mL Erlenmeyer flasks) was inoculated with 1 mL of standard overnight cultures (initial number of cells 10^4) and incubated at 50°C in a orbital shaker (Thermo Forma, Ohio, USA) operated at 150 rpm for 264 h. Triplicate flasks were withdrawn at regular intervals. Contents were analyzed for pH and growth, based on viable cell counts (CFU mL^{-1}), and then centrifuged (HERMLEZ 382 K, Wehingen, Germany) at 15,500 g for 15 min at 4°C. The cell free supernatant obtained was used as crude enzyme preparations. The experiments were carried out in duplicates and the results were expressed as mean values.

2.3. Enzymes assay

The cellulolytic enzyme activities were determined using the dinitrosalicyclic acid method [11], which measures reducing sugars. The reaction mixture containing 0.5 mL of 1% (w/v) substrate solution (carboxymethylcellulose sodium salt or avicel, PH-101) prepared in 10 mM sodium phosphate buffer, pH 7.5, and 0.5 mL of appropriate concentration of enzyme solution, was incubated at 70°C. After 10 min of reaction, 1 mL of dinitrosalicyclic acid reagent was added and the mixture was then boiled in a water bath for 5 min. The resulting samples were cooled to room temperature, and absorbance was measured at 540 nm. When the activity was tested on avicel as substrate, the assay tubes were shaken during the course of the assay, to keep the substrate suspended. One unit (U) of activity towards the substrates mentioned above was defined as 1 μmole of glucose equivalent released per minute under the conditions described above, using a glucose standard curve. Appropriate controls were conducted in parallel with all assays. Enzyme blanks containing 0.5 mL of 10 mM sodium phosphate buffer and 0.5 mL of 1% (w/v) substrate solution were also run. To exclude the background of reducing sugars found in the enzyme supernatant from the results, a substrate blank was also run containing 0.5 mL of 10 mM sodium phosphate buffer and 0.5 mL enzyme solution. The absorbance values of the enzyme blank sets and of the substrate blank were subtracted from the absorbance of the activity assay. All sample analyses were run in triplicate, while the blanks were run in duplicate.

2.4. Partial purification of enzymes

Solid ammonium sulfate at 60% saturation was added to the crude enzymes to obtain partially purified enzymes by submerged fermentation, with constant stirring at room temperature. The precipitate was collected by centrifugation at 12,000 g for 20 min at 4°C. This precipitate was dissolved in 10 mM sodium phosphate buffer, pH 7.5, and dialyzed overnight in the same buffer for 24 h with three changes every 8 h. The dialyzed enzyme was then concentrated by lyophilization and stored at -20°C.

2.5. Determination of total soluble exocell protein

Concentration of total soluble exocell protein was determined by Lowry's method, modified by Peterson [12]. Bovine serum albumin was used as a standard.

2.6. Effect of pH in the activity and stability of cellulases

The effect of pH on cellulase activities was estimated by varying the pH of the assay reaction mixture using the following buffers (50 mM) in the enzyme assay mixture: citrate phosphate, pH 6.0–6.5, sodium phosphate, pH 7.0–7.5, Tris–HCl, pH 8.0–9.0, and borax-NaOH, pH 9.5–11.0. The effect of pH on stability of the enzymes was determined by pre-incubating the enzymes with the buffer of appropriate pH (6.0–11.0) for 3 h at room temperature, whereupon residual activity was assayed. Relative activity was determined by considering maximum activity as the standard reference.

2.7. Effect of the temperature in the activity and stability of cellulases

The effect of temperature on the enzyme activities was determined by performing the standard assay procedure within 30–90°C. The thermal stability of cellulases secreted by *Bacillus* sp. SMIA-2 was tested by determining the enzyme activity remaining after incubation of the enzymes at 30–90°C for 1 h in a constant-temperature water bath. The residual activities were determined under optimum pH and temperature conditions as described above. Relative activity was determined by considering maximum activity as the standard reference.

2.8. Activity towards different substrates

In order to determine the activity of the dialyzed fraction of a sugarcane bagasse culture towards different substrates, 1% (w/v) solutions of several substrates (oalt spelts xylan, carboxymethylcellulose, avicel, microgranular cellulose and soluble starch) were prepared in 10 mM sodium phosphate buffer, pH 7.5. Volumes of 0.5 mL of each substrate were separately incubated with 0.5 mL of appropriate concentration of enzyme solution at 70°C (except amylase, which was incubated at 90°C) for 10 min. Activities were expressed as μmole of reducing sugar (glucose or xylose) equivalent released per minute. All experiments were conducted in triplicates, and results expressed as average values.

2.9. Detection of proteases

Protease activity was assessed in triplicate by measuring the release of trichloroacetic-acid soluble peptides from 0.2% (w/v) azocasein in 0.2 M Tris–HCl buffer (pH 8.5) at 70°C for 10 min. The 1 mL reaction was terminated by the addition of 0.5 mL of 15% (w/v) trichloroacetic acid. Then, contents were centrifuged at 15,000 g for 5 min, after cooling. One enzyme activity unit (U) was defined as the amount of enzyme required to produce an increase in absorbance at 420 nm equal to 0.1 in 60 min.

2.10. Effect of some surfactants and oxidizing agents

Appropriate concentration of enzyme was pre-incubated (final concentration) with 0.25% (w/v) and 0.5% (w/v) sodium dodecyl sulfate, linear alkylbenzene sulfonate, RENEX-60, RENEX-95, triton X-100 and hydrogen peroxide at 40°C for 15 and 30 min. Activity (avicelase) was measured under standard conditions.

2.11. Stability with various commercial detergents

The detergent brands used were Ariel®, Tixan®, Omo®, Biz®, Cheer®, and Tide®. They were diluted in double distilled water to a final concentration of 7 mg·mL^{-1} to simulate washing conditions and heated at 100°C for 15 min to inactivate the enzymes that could be part of their formulation. After that, an appropriate concentration of enzyme was added in solution and incubated at 40°C for 60 min. Aliquots were taken at different time intervals, and the residual cellulase (avicelase) activity was measured under standard conditions and compared with the control sample without any detergent.

3. Results

Bacillus sp. SMIA-2 produced cellulases (avicel-hydrolyzing enzymes — avicelases), when growing in liquid medium containing sugarcane bagasse as a carbon source (Fig. 1). To improve enzyme activity, the culture medium was enriched with corn steep liquor as nitrogen source. Corn steep liquor is a source of amino acids, vitamins and metal ions and thus was used in the culture medium in place of meat and yeast extracts, which are comparatively costlier nitrogen sources. The secreted avicelase exhibited maximum activity (0.83 U mL^{-1}) in 120 h of culture time.

Culture filtrates also showed carboxymethylcellulase (CMCase) activity. The maximum enzyme activity (0.29 U mL^{-1}) extended until the 168th h of culture time, when then, started to decline.

Changes in medium pH were also followed during cultivations of *Bacillus* sp. SMIA-2 on sugarcane bagasse. pH increased from an initial value of 7.1 to 8.5 at the end of the fermentation period.

The supernatant from cultures of *Bacillus* sp. SMIA-2 grown in sugarcane bagasse containing 1.01 mg·mL^{-1} of protein was used as crude enzyme solution and subjected to partial purification by ammonium sulfate precipitation, followed by dialysis. This dialyzed fraction was lyophilized and called partially purified enzymes.

A pH range between 4.0 and 11.0 was used to study the effect of pH on enzyme activities. Avicelase and CMCase presented optimum activity at pH 7.5 and 8.0, respectively (Fig. 2a). Testing pH stability of the enzymes produced by *Bacillus* SMIA-2 revealed that maximum stability of avicelase and CMCase was observed at a pH range between 6.5–8.0 and 7.0–9.0, respectively, where they retained more than 70%

of their maximum activities, after incubation at room temperature for 3 h within this pH range (Fig. 2b).

The avicelase and CMCase from *Bacillus* sp. SMIA-2 grown in SCB were significantly active in the temperature range tested (Fig. 3). Enzyme activities increased with temperature within 30°C to 70°C (Fig. 3a). A reduction in enzyme activities was observed above 70°C. The optimum temperature of avicelase was 70°C. Moreover, at 80°C the dialyzed enzyme still retained 90% of the maximum activity. The optimum temperature of the CMCase was also at 70°C, and activity levels of about 40% were still detected at 90°C.

The effect of the temperature in the stability of enzymes is shown in Fig. 3b. The enzymes remained 100% stable at up to 60°C (for 1 h), and started to undergo denaturation thereafter.

The dialyzed fraction of a sugarcane bagasse culture also displayed activity on starch, oalt spelts xylan, and polygalacturonic acid, and, to a lesser extent, on microgranular cellulose (Table 1). *Bacillus* sp. SMIA-2 cultures also released proteases (38.25 U/mg protein) into the culture medium.

Testing detergent compatibility of the avicelase produced by *Bacillus* SMIA-2 revealed that the enzyme retained more than 70% of its activity after 30 min of incubation at 40°C in the presence of the detergent brands Ultra Biz®, Omo®, and Tide® (Fig. 4). In addition, the enzyme retained more than 75% activity after incubation for 30 min at 40°C with 0.25% and 0.5% (w/v) sodium dodecyl sulfate, RENEX-95, RENEX-60, and more than 60% of its activity with 0.25% and 0.5% (w/v) linear alkylbenzene sulfonate. However, the incubation in the presence of 0.25% and 0.5% (w/v) TritonX-100 and H_2O_2 drastically inhibited enzyme activity (Table 2).

4. Discussion

An important consideration in fermentation processes is the cost of the carbon source, which contributes significantly to overall production expenses. Accordingly, sugarcane bagasse, which contains amorphous cellulose, crystalline cellulose and hemicellulose (which consists predominantly of xylan) was examined as substrate to cellulases production by a thermophilic soil bacterium *Bacillus* sp. SMIA-2.

Bacillus sp. SMIA-2 grew rapidly in cultures shaken at 50°C, and expressed a promising level (0.83 U/mL) of cellulase (or avicel-hydrolyzing enzymes — avicelases) in 120 h of culture time.
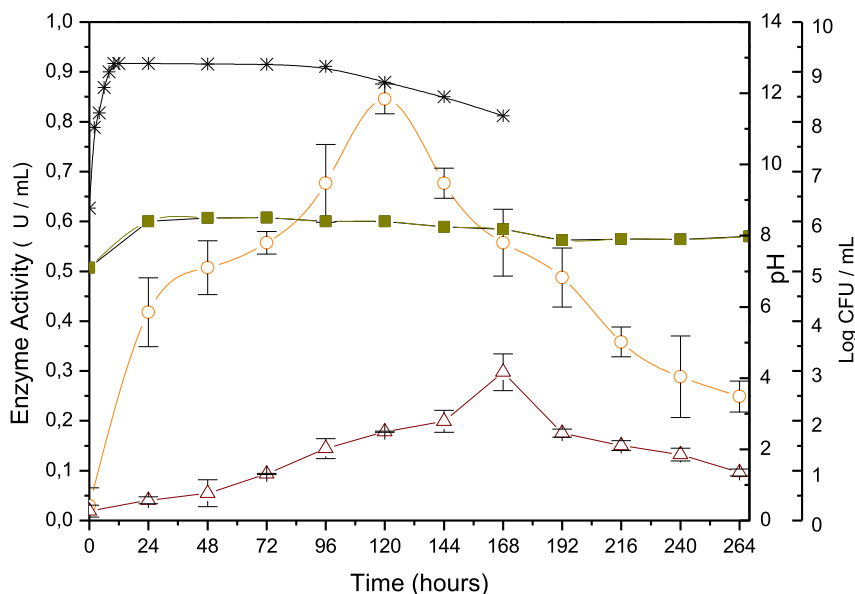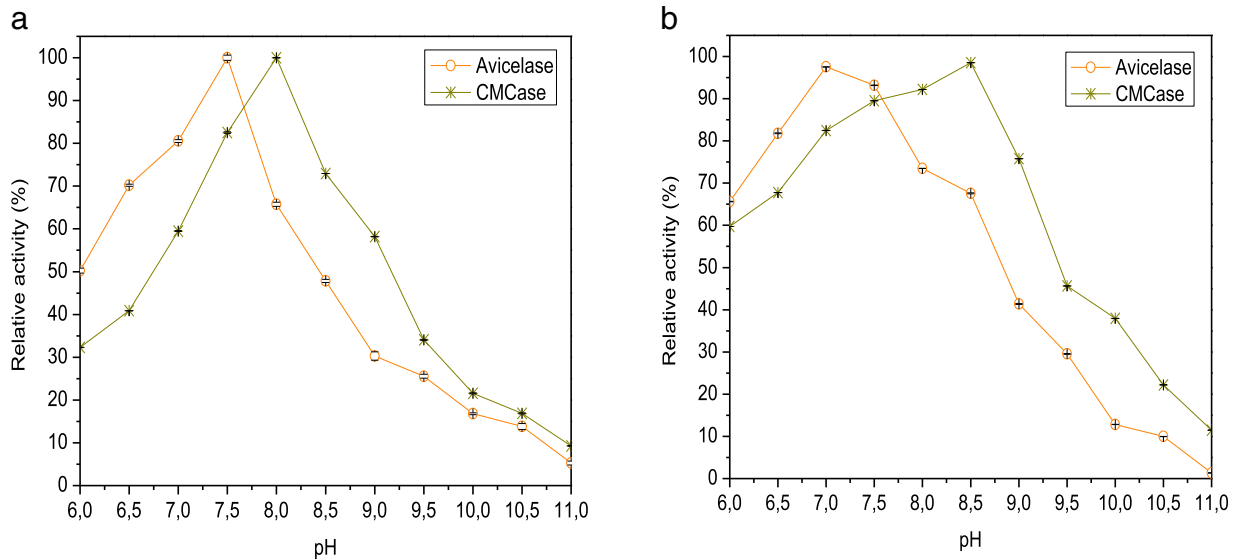

Fig. 1. Growth (*), pH (■), avicelase (○) and CMCase (△) secreted by *Bacillus* sp. SMIA-2, when grown in liquid medium containing sugarcane bagasse and corn steep liquor for 264 h at 50°C. Results are means of two independent determinations. Bars correspond to standard deviation.
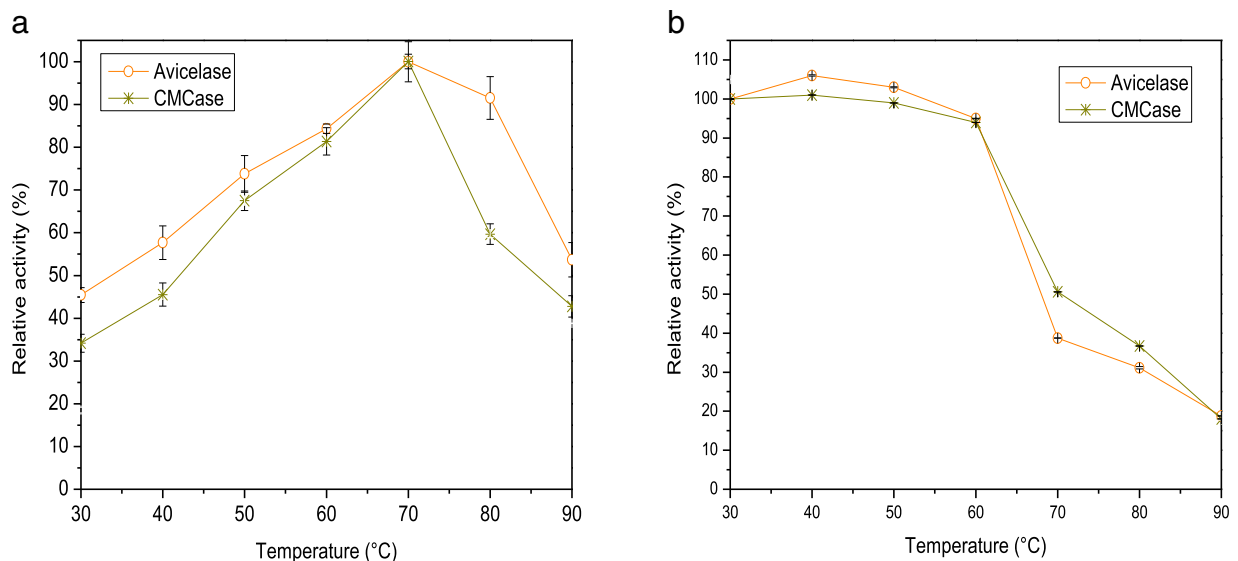
Fig. 2. Effect of varying pH values on cellulase activity (a) and stability (b). Relative activity is expressed as a percentage of the maximum (100% of avicelase activity = 4.99 U/mg protein; 100% of CMCase activity = 2.55 U/mg protein).

Makky [4] described avicelase production by a thermophilic *Geobacillus stearothermophilus* isolated from soil using sugarcane bagasse treated and untreated under optimized conditions. The maximum enzyme activity expressed was 1.99 IU/mL and 1.06 IU/mL, in treated and untreated substrates, respectively.

Avicelase activity is a measurement of the ability of the crude enzyme preparation to hydrolyze microcrystalline cellulose. The culture filtrates also showed ability to hydrolyze carboxymethylcellulose, which is generally considered a substrate for measurements of CMCase activity. Maximum CMCase activity extended until the 168th h, when the culture reached the death phase. The activity of CMCase was lower, when compared with avicelase, suggesting that the cell-free supernatants from *Bacillus* sp. SMIA-2 cultures containing sugarcane bagasse probably contained high exoglucanase levels. This result was unexpected, as the most *Bacillus* had higher activity with carboxymethylcellulose. Similar results were reported by Beukes and Pletschke [13], in that the cellulosomal cellulases from *Bacillus*

megaterium has both CMCase and avicelase activity; however, the 'cellulases' had a higher activity with avicels. The mechanisms by which different types of cellulases enhance each other's activities are complex and not completely understood, and the published data are often inconsistent [14].

Kim and Kim [15] reported that a moderately thermophilic *Bacillus* K-12 produced a large amount of cellulase components containing avicelase, xylanase, CMCase and FPase, when grown in avicel medium at 50°C. According to these authors, avicelase and FPase in the culture broth increased rapidly, reaching maximum levels on the 8th day of cultivation. Rastogi et al. [16] reported the maximum production of CMCase by cellulose-degrading bacteria (DUSELG12) on d 10, when the culture reached the death phase, while maximum cellulase activity was observed on day 7, at the end of exponential growth phase, after which cellulase activity decreased with time. A 10-d incubation period was essential for cellulase production in *Bacillus* sp. [17].

Fig. 3. Effect of varying temperature values on cellulase activity (A) and stability (B). Relative activity is expressed as a percentage of the maximum (100% of avicelase activity = 4.99 U/mg protein; 100% of CMCase activity = 2.55 U/mg protein).

Table 1
Enzymatic activity on different substrates.

| Substrates	Enzymatic activity (U/mg protein)
Avicel	4.99 ± 0.09
Carboxymethycellulose	2.55 ± 0.16
Oalt spelts xylan	4.28 ± 0.13
Microgranular cellulose	2.62 ± 0.29
Polygalacturonic acid	4.14 ± 0.33
Soluble starch	5.87 ± 0.03

Thus, *Bacillus* sp. SMIA-2 might become a useful source of economically important lignocellulose-degrading enzymes, with potential applications in the industry.

pH variation was also evaluated during cultivations of *Bacillus* sp. SMIA-2 on sugarcane bagasse. The profile showed an increase, as cells started to grow. The increase in pH may be attributed to the presence of exocell proteins, because intermediate pH values tend to increase, if organic amino compounds are deaminated.

The supernatant from cultures of *Bacillus* sp. SMIA-2 grown in sugarcane bagasse and corn steep liquor (120 h) contained 1.01 mg mL^{-1} of protein. The proteins may partly have originated from the complex lignocellulosic biomass or from cells following lysis, and may also constitute different enzymes, secreted to digest the substrate. While expensive purification procedures may be required for their removal, most industrial enzymes are consumed in the presence of impurities that originate from culture supernatants. Thus, the cellulases secreted by *Bacillus* sp. SMIA-2 were not purified, beyond ammonium sulfate precipitation followed by dialysis.

Testing the pH-dependence of enzymes present in the dialyzed fraction from cultures of *Bacillus* sp. SMIA-2 grown in sugarcane bagasse and corn steep liquor reveled that avicelase exhibited optimum activity at pH 7.5, which was a little higher than that reported by Makky [4] for a strain of *G. stearothermophilus*, which was pH 7.0. Regarding CMCase, the enzyme showed optimum activity at pH 8.0, and was higher than that found for endoglucanase from *Bacillus pumilus* EB3 [3].

The optimum temperature for avicelase and CMCase activity was 70°C, which was similar to the values reported in the literature for other *Bacillus* strains [18,19,20]. At 80°C, the activity of avicelase was still suitable, but CMCase activity decreased sharply. Tai et al. [21] showed that CMCase activity in *Geobacillus* sp. dropped immediately, even with a slight increase of temperature beyond 70°C, indicating that the enzyme was highly sensitive to temperatures higher than that.
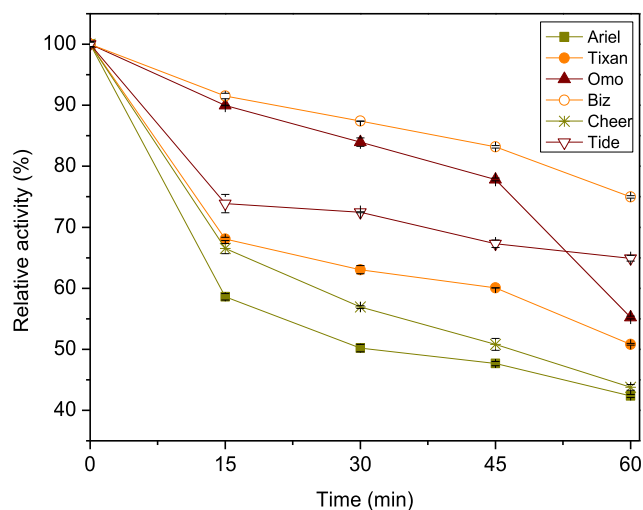
Fig. 4. Stability of avicelase from *Bacillus* sp. SMIA-2 with different commercial detergents. Relative activity is expressed as a percentage of the activity level in the absence of detergents (100% of avicelase activity = 4.99 U/mg protein).

Table 2
Effect of various inhibitors and surfactants on avicelase from *Bacillus* sp. SMIA-2.

Residual avicelase activity (%)[a]		
	Incubation time (min)	
	15	30
Inhibitors/surfactants		
Control (no addition)	100.0 ± 0.11	100.0 ± 0.09
Sodium dodecyl sulfate 0.25%	97.1 ± 0.11	95.1 ± 0.30
Sodium dodecyl sulfate 0.5%	91.8 ± 0.21	87.7 ± 0.07
Renex−95 0.25%	98.5 ± 0.13	93.5 ± 0.09
Renex−95 0.5%	88.1 ± 0.27	83.9 ± 0.04
Renex−60 0.25%	86.1 ± 0.00	75.5 ± 0.02
Renex−60 0.5%	80.3 ± 0.24	77.1 ± 0.03
Linear alkylbenzene sulfonate 0.25%	72.0 ± 0.03	64.8 ± 0.21
Linear alkylbenzene sulfonate 0.5%	66.7 ± 0.10	61.1 ± 0.13
Triton x−100 0.25%	31.7 ± 0.09	27.1 ± 0.10
Triton x−100 0.5%	22.0 ± 0.11	21.4 ± 0.13
H$_2$O$_2$ 0.25%	6.4 ± 0.08	4.0 ± 0.02
H$_2$O$_2$ 0.5%	4.3 ± 0.04	2.6 ± 0.01

[a] Residual activity was expressed as a percentage of control. 100% of enzyme activity = 4.99 U/mg protein.

The avicelase and CMCase present in the dialyzed fraction retained their maximum activities after incubation for 1 h at temperatures between 40–60°C; however, the enzymes lost about 50% of their maximum activities after incubation for 1 h at 70°C. This could be associated with the removal of ligand and/or proteins that have a protective effect on the crude enzyme. It has been reported that the removal of these components could decrease the thermostability or affinity for the substrate [22]. In addition, some but not all thermophiles are known to contain metabolites that strongly stabilize proteins, although it is not certain whether this is indeed their primary function [23].

Apart from these results, the thermostability of cellulases competed favorably with the values reported for the genus *Bacillus*. A thermostable cellulase was found in newly isolated *Bacillus subtilis* DR, extracted from a hot spring. The high temperature environment allowed the production of a thermostable endocellulase CelDR, with an optimum yield at 50°C. It was found to retain 70% of its maximum activity (CMCase) after incubation at 75°C for 30 min [24]. Furthermore, a salt-activated endoglucanase was isolated from another *Bacillus* strain, alkaliphilic *Bacillus agaradhaerens* JAM-KU023, which was shown to have increased optimal thermostability from 50°C to 60°C with the addition of 0.2 M NaCl, and optimal pH range from 7–9.4 [25]. Thermostable enzymes such as cellulases are advantageous for some applications, because higher processing temperatures can be employed, with consequent faster reaction rates, improved hydrolysis of cellulosic substrates, and reduced incidence of microbial contamination from mesophilic organisms [26].

As thermoactivity and pH stability of enzymes are of great importance in industrial uses, enzymatic properties presented by cellulases suggest the potential use of these enzymes in various industrial applications, such as in the detergent industries. In general, all currently used enzymes that are detergent compatible are also alkaline and thermostable by nature [27], because the pH of laundry detergents is generally in the range of 9.0–12.0 and the range of temperatures used for washing (50–70°C).

The dialyzed fraction of a sugarcane bagasse culture displayed higher activity with avicels. The dialyzed fraction also displayed activity on xylan and polygalacturonic acid. Many different enzymes are required in order to achieve effective degradation of complex plant biomass. Some organisms express free enzymes, while others produce a large, extracellular multi-enzyme complex such as the cellulosome [28].

Bacillus sp. SMIA-2 cultures also released proteases (38.25 U/mg protein) and α-amylases into the culture medium. It is known from the previous studies that *Bacillus* SMIA-2 produces extracellular

proteases [29] and α-amylases [30] during the post-exponential growth phase under most growth conditions. Proteases are the main enzyme class used in the formulation of enzymatic detergent, followed by amylases. However, proteases have the ability to degrade azocasein and other proteins, and could potentially cause a decrease in cellulolytic enzyme activities, but the cellulases secreted by *Bacillus* sp. SMIA-2 were resistant to protease digestion. According to Caparrós et al. [31], cellulases from *Bacillus* species generally show good compatibility with subtilisin proteases, which is interesting for detergent applications.

The compatibility of cellulase (avicelase) with some detergents commonly used was tested with a view to exploiting the enzyme in detergent industry. Addition of cellulases and proteases in the detergent compositions is a new trend followed by many detergent industries in recent years. Alkaline cellulases present in detergent composition can pass through the interfibril spaces easily, effectively removing stains from textiles. Additionally, cellulases process cellulose fibrils, and impart color brightness and smoothness to clothes, even after repeated washing [1].

The compatibility of an enzyme varied for each laundry detergent, with higher stability being observed in the presence of Ultra Biz®. The opposite was reported with Ariel®. These findings suggest that the performance of an enzyme in detergents depends on a number of factors, including the compounds used in formulations. In fact, the enzyme was stable in sodium dodecyl sulfate and RENEX-95 and was inhibited by TritonX-100 and H_2O_2.

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5. Conclusion

The wild *Bacillus* sp. strain SMIA-2 produced promising level of cellulases under thermophilic conditions from submerged cultures containing sugarcane bagasse supplemented with corn steep liquor. These cellulases could release glucose from soluble and insoluble crystalline cellulose. They also showed broad temperature and pH stabilities, and were not inhibited by some common surfactants. This indicates the potential applications of the cellulase produced by *Bacillus* sp. SMIA-2 in industry, such as in detergents.

Conflict of interest

There is no conflict of interest.

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