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Purification and characterization of a thermostable alkaline cellulase produced by *Bacillus licheniformis* 380 isolated from compost

ÉVILIN G. DE MARCO[1], KARINA HECK[2], EMERSON T. MARTOS[3], SUELI T. VAN DER SAND[1*]

[1]Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Microbiologia Agrícola e do Ambiente, Rua Sarmento Leite, 500, 90050-170 Porto Alegre, RS, Brazil
[2]Universidade Federal de Lavras, Programa de Pós-Graduação em Tecnologias e Inovações Ambientais, Rua Sylvio Menicucci, 1001, 37200-000 Lavras, MG, Brazil
[3]Instituto Superior de Ciências Aplicadas, Rodovia Deputado Laércio Corte, 3000, Chácara Boa Vista da Graminha, 13482-383 Limeira, SP, Brazil

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ABSTRACT

During composting processes, the degradation of organic waste is accomplished and driven by a succession of microbial populations exhibiting a broad range of functional competencies. A total of 183 bacteria, isolated from a composting process, were evaluated for cellulase activity at different temperatures (37, 50, 60, and 70°C) and pH values. Out of the 22 isolates that showed activity, isolate 380 showed the highest cellulase activity. Its ability to produce cellulase was evaluated in culture medium supplemented with carboxymethyl cellulose, microcrystalline cellulose, wheat straw, and rice husk. The culture medium supplemented with carboxymethyl cellulose induced higher enzyme activity after 6 hours of incubation (0.12 UEA mL^{-1} min^{-1}). For wheat straw and rice husk, the results were 0.08 UEA mL^{-1} min^{-1} for both, while for microcrystalline cellulose, 0.04 UEA mL^{-1} min^{-1} were observed. The highest carboxymethyl cellulase activity was observed at 60°C (0.14 UEA mL^{-1} min^{-1}) for both crude and partially purified enzyme after 30 and 120 min of incubation, respectively. Alkalinization of the medium was observed during cultivation in all substrates. The cellulase had a molecular mass of 20 kDa determined by SDS-Page. Isolate 380 was identified as *Bacillus licheniformis.* This work provides a basis for further studies on composting optimization.

Key words: alkalophilic enzyme, carboxymethyl celullase, compost, high temperature.

INTRODUCTION

Cellulose is considered the most available biomass around the world and a vital, sustainable, and renewable energy source. It is also the most recalcitrant biomass polymer and difficult to degrade. Synthetic enzymes can catalyze the metabolic conversion of cellulose to glucose; however, research efforts to replace synthetic enzymes by microbial enzyme processes are currently being made to reduce costs and mitigate the industrial and environmental impacts. Pulp products are found in biofuels, biofertilizers, and products of the textile industry (Ladeira et al. 2015, Gaur and Tiwari 2015).

Correspondence to: Sueli Teresinha Van Der Sand
E-mail: svands@ufrgs.br

Plant biomass waste consists almost entirely of lignocellulose, which is formed by three types of polymers, namely cellulose, hemicellulose, and lignin. Cellulose is a linear polymer composed of D-glucose linked by β-1,4 glucoside bonds (Béguin and Aubert 1994, Lynd et al. 2002). The cellulose enzyme system usually comprises three cellulolytic enzyme classes which hydrolyze the cellulose: endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91), and cellobiases (EC 3.2.1.21) (Foster and Whiteman 1992, Rabonovich et al. 2002). These enzymes are widely used to substitute chemical compounds mainly in the pulp and paper industry and in wastewater treatment (Bhat 2000, Acharya and Chaudhary 2012).

Microorganisms are employed directly for bioaugmentation, which is widely used in waste treatment plants (Martin-Ryals et al. 2015). Besides these applications, it is necessary to know the enzymatic capacity of the microorganisms used in any environmental treatment. Cellulases are usually produced by fungi, such as *Trichoderma* sp., *Aspergillus* sp. and *Lentinula* sp. (Bhat 2000, Carvalho et al. 2016), however, these cellulases do not exhibit thermal stability, and environmental induction is necessary for their production. On the other hand, the enzymes produced by bacteria are constitutive (Acharya and Chaudhary 2012). Many species of the genus *Bacillus* have been studied in terms of the production of thermostable cellulases, such as *B. pumilus* (Christakopoulos et al. 1999), *B. circulans* (Waeonukul and Ratanakhanokchai 2007), *B. subtilis* (Shabeb et al. 2010), *B. licheniformis* (Acharya and Chaudhary 2012) and *B. vallismortis* (Gaur and Tiwari 2015).

Composting is a method of waste treatment and comprises organic matter biodegradation mediated by the activities of microbial enzymes. The process occurs under aerobic conditions and through polymer hydrolysis and monomer oxidation, following the use of sugars as a carbon source. Humic and fulvic acids are produced at the end of the process after the mineralization of carbon dioxide. The microbial community profile is defined according to each phase related to increasing or reducing temperatures and the compounds produced in the previous degradation step (Amore et al. 2013). Complex polymers such as proteins, cellulose, lignin, and other recalcitrant molecules are degraded mainly during the thermophilic phase (Sánchez-Monedero et al. 2001), which also contributes to eliminate or decrease pathogenic microorganisms along the composting process (Palmisano and Barlaz 1996, Heck et al. 2013).

The composting process offers an opportunity for the screening of enzymes produced by microorganisms that optimize the biodegradation of recalcitrant organic compounds such as lignocellulosic structures (Lópes-González et al. 2014, Kinet et al. 2015). The aim of this study was therefore to evaluate and characterize the production of carboxymethyl cellulases (endoglucanases) by bacteria isolated from the thermophilic phase of a composting process by applying different carbon sources as substrates in a broad range of temperature and pH conditions.

MATERIALS AND METHODS

BACTERIAL ISOLATES

The bacteria evaluated in this study were isolated from a compost windrow made of organic domestic waste, pruning residue, and sewage sludge from a domestic wastewater treatment plant (Heck et al. 2015). The microorganisms were selected based on their capacity to grow in the thermophilic phase of the composting process; the selection was based on their ability to hydrolyze cellulose using different carbon sources as substrate.

ENZYME INDEX IN SOLID MEDIUM

Cellulolytic activity was evaluated using the culture medium proposed by Ramachandra et al. (1987), supplemented with 0.5% carboxymethyl cellulose (CMC). The isolates were inoculated, using the spot inoculation method, onto plates containing the culture medium and incubated at 37, 50, and 60°C for four days and at 70°C for three days. After growth, the plates were stained with Lugol solution (1%) for 3-5 minutes for the visualization of CMC hydrolysis zones (Kasana et al. 2008). Halos were measured for subsequent selection of cellulolytic microorganisms with a high potential for hydrolysis activity. The enzymatic activity index (IE) was calculated according to Hankin and Anagnostakis (1975).

ENZYME ACTIVITY IN SUBMERGED CULTURES

Isolates with cellulase activity in solid medium at all tested temperatures were cultured in a submerged culture. Conical flasks containing 50 mL of broth medium (Ramachandra et al. 1987) supplemented with CMC 0.5% (m/v) were inoculated with 10% of an inoculum culture of each isolate (10^8 cells mL^{-1}, OD 0.5, 570 nm), and incubated under agitation at 150 rpm at 50, 60, and 70°C for 12 hours. Enzymatic activity was determined every two hours. The culture was centrifuged at 13.000 x g for 15 min to separate the cells and the supernatant was considered the enzyme crude extract. A glucose standard curve was established to determine reducing sugar concentrations, according to Nelson (1944) and Somogy (1952). One unit of enzymatic activity (UEA) was considered the unit of enzymatic activity equivalent to the release of 1 µmol glucose mL^{-1} min^{-1}. All assays were performed in triplicate.

ENZYME ACTIVITY IN THE PRESENCE OF DIFFERENT SUBSTRATES IN SUBMERGED CULTURE

The strain that showed the best result in liquid medium was evaluated for the production of cellulase using different sources of cellulose substrate, such as CMC, microcrystalline cellulose, crushed rice husk and wheat straw. The flasks containing the liquid culture medium (50 mL) supplemented with 0.5% of different cellulose substrate as carbon source were incubated under agitation at 150 rpm at 50°C for 12 hours. Every two hours, we determined the numbers of viable cells and endospores, as well as pH values and enzyme activity. All assays were performed in triplicate.

ENZYME PURIFICATION

For enzyme purification, the supernatant was saturated overnight with ammonium sulphate at 60-80%, at a temperature of 4°C. Subsequently, the precipitate was collected by centrifugation at 10,000 x g for 10 min at 4°C, resuspended in phosphate buffered saline (PBS) (pH 7.2), and kept at 4°C. The suspension was applied to a Sephadex G-100 gel filtration column (2.5 x 40 cm), equilibrated with PBS buffer (pH 7.2), and eluted in a gradient of NaCl (0–1 mol L^{-1}). Twenty fractions of 1.0 mL were collected and each fraction was submitted to an enzyme activity assay. The fractions with higher activities were mixed and kept for further assays.

ACTIVITY AND THERMIC STABILITY OF CRUDE EXTRACT AT DIFFERENT PH VALUES

The crude extract was submitted to enzymatic activity and thermic stability by incubating 2.0 mL of the extract in a water bath at 30, 40, 45, 50, 55, 60, 70 and 80°C with buffer solutions (0.2M Na_2HPO_4 + 0.1M $C_6H_8O_7$ for pH 4-8; 0.1M Tris-HCl for pH 9; 0.05M $NaHCO_3$ + 0.1M NaOH for pH 10). After 30 min, an aliquot was collected for

enzyme activity determination. Culture medium without inoculation was used as a control. The concentration of soluble protein was determined using the method describe by Bradford (1976).

SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

The crude extract was analyzed by SDS-PAGE gel electrophoresis with 5% staking gel and 12% polyacrylamide. An aliquot of 35 µL of the sample was heated at 100°C for 5 min and applied to the gel; electrophoresis was performed at 50 mA for 2 hours. The gel was stained with Coomassie Brilliant Blue G250 (Sigma-Aldrich) in methanol-acetic acid-water (5:1:5, v/v). Decolorization was performed in 7% acetic acid.

ISOLATE IDENTIFICATION

Morphological and physiological tests were carried out according to Holt et al. (1994). Whole bacterial genomic DNA was extracted following the method described by Lin et al. (2010). The 16S rDNA gene was amplified using the primers 27F1 AGAGTTTGATCCTGCTCAG and 1494Rc TACGGCTACCTTGTTACGAC (Neilan et al. 1997). The reaction was performed at 1 × reaction buffer, 0.2 mM of each dNTP, 3 mM of $MgCl_2$, 1.5 U of Platinum® Taq DNA polymerase, 10 pmol of each primer, 100 ng of DNA, and ultrapure MilliQ water for a final reaction volume of 25 µL, in a Gene Amp PCR System 9700 thermocycler (Applied Biosystems). The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 94°C for 10 s, annealing at 50°C for 20 s, elongation at 72°C for 1 min, final extension at 72°C for 7 min. After the reaction, 1 µL of amplified fragments was mixed with 2.0 µL of loading buffer to verify integrity by electrophoresis in 1% agarose gel. The quantification of fragments was compared with Lambda DNA/EcoRI + HindIII DNA size standard (Promega) documented by the Kodak Gel Logic 212 system. Afterwards, the fragments were cloned into pGEM®-T Easy Vector Systems cloning vector as recommended by the manufacturer. *Escherichia coli* DH5α cells were transformed with the constructed clones (Sambrook et al. 1989). Sanger sequencing reaction was performed with BigDye XTerminator (GE Healthcare) using the vector primers M13 F/R and the internal primers 307 F/R, 704 F/R, and 1114 F/R (modified by Lane 1991). Its products were inserted into an *ABI PRISM® 3100 Genetic Analyzer* capillary sequencer (Applied Biosystems). The quality of the reads generated was assessed using the software package Phred/Phrap/Consed (Ewing et al. 1998, Ewing and Green 1998, Gordon et al. 1998). The consensus sequence obtained was compared with other sequences available in the GenBank/NCBI database using the BLAST tool (Altschul et al. 1997). The sequence has been deposited at GenBank/NCBI under the accession number MF099893.

PHYLOGENETIC ANALYSES

The nucleotide sequences obtained from the Sanger sequencing and the reference sequences retrieved from GenBank were aligned using CLUSTAL W (Thompson et al. 1994). All sequences were trimmed (sequence data matrix) and used to reconstruct phylogenetic trees. The 16S rDNA gene phylogenetic trees were inferred using the maximum-likelihood (ML) reconstructed using the MEGA program package, version 7 (Kumar et al. 2016), using Kimura's two-parameter model of sequence evolution. The robustness of the phylogenetic tree was estimated via bootstrap analysis using 1,000 resamplings. The tree was visualized with FigTree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree).

RESULTS

Composting of organic waste, urban pruning, and sewage sludge allowed the isolation of 183

bacterial isolates in the thermophilic phase. Among these, 22 were able to hydrolyze CMC on agar when incubated at 37°C, 15 showed cellulolytic activity when incubated at 50°C, seven performed cellulolytic activity when incubated at 60°C, and three showed cellulolytic activity when incubated at 70°C. Furthermore, the isolates that showed activity at 70°C were also able to hydrolyze CMC under all tested temperatures. Comparing the three isolates that presented the best profile for CMCase activity (380, 381, and 382), we observed the largest halo produced by isolate 380 at all temperatures tested. Under incubation at 70ºC, isolate 380, in contrast to isolates 381 and 382, did not show activity reduction (Table I). Therefore, isolate 380 was selected for further enzymatic activity assays.

These isolates were collected at the end of the thermophilic phase of the composting process and at the beginning of the maturation phase, at an average temperature of 46ºC (Heck et al. 2015). Thus, we selected the temperature of 50ºC as the most suitable temperature for halo production in the solid cultures (Table I).

The three isolates showed enzymatic activity in submerged cultures supplemented with carboxymethyl cellulose at a range from 0.04 to 0.12 UEA mL^{-1} min^{-1} (Table II). Activity peaks for each isolate started between four and six

TABLE I
Isolates producers of CMCase in solid medium supplemented by CMC 0.5% (m/v) and hydrolysis halos (mm ± SD*).

	Halo (mm ± SD) for Temperatures of Incubation (°C)			
ISOLATE	**37**	**50**	**60**	**70**
71	40 ± 2.8	35 ± 3.5	-	-
72	35 ± 3.5	12.5 ± 3.5	-	-
75	21 ± 7.0	-	-	-
92	47 ± 4.2	39 ± 6.3	10 ± 2.8	-
103	35 ± 0	20 ± 1.4	-	-
104	35 ± 5.6	15 ± 4.2	-	-
106	28 ± 11.3	4.0 ± 0	-	-
135	28 ± 2.8	-	-	-
148	52 ± 2.8	44.5 ± 0.7	-	-
269	22 ± 3.5	-	-	-
273	20 ± 5.6	-	-	-
274	6.5 ± 2.1	-	-	-
291	48 ± 11.3	12 ± 2.8	-	-
297	19 ± 6.3	-	-	-
302	46 ± 0	31 ± 4.2	15 ± 5.0	-
347	45 ± 2.1	-	-	-
351	30 ± 5.6	25 ± 7.0	-	-
379	46 ± 3.5	59 ± 5.0	10 ± 7.0	-
380	54 ± 4.2	60 ± 2.8	23.5 ± 2.1	20 ± 2.8
381	47 ± 2.8	59 ± 1.4	26 ± 1.4	6.0 ± 2.8
382	46 ± 8.4	37.5 ± 2.1	22 ± 0	14 ± 3.5
387	52 ± 4.2	52 ± 2.8	26 ± 1.4	-

*SD - Standard deviation.

TABLE II
CMCase enzyme activity (UEA mL^{-1} min^{-1}) for isolates selected and cultured at 50°C in submerged culture supplemented with 0.5% of CMC.

Time of incubation	380	381	382
2 h	0.04 ± 0.008	0.06 ± 0.02	0.06 ± 0.04
4 h	0.06 ± 0.01	0.06 ± 0.02	0.08 ± 0.01
6 h	0.12 ± 0.008	0.08 ± 0.01	0.08 ± 0.01
8 h	0.08 ± 0.02	0.08 ± 0.01	0.08 ± 0.008
10 h	0.08 ± 0.03	0.08 ± 0.01	0.06 ± 0.01
12 h	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01
24 h	0.06 ± 0.01	0.04 ± 0.02	0.04 ± 0.009

TABLE III
Enzyme activity (UEA mL^{-1} min^{-1}) for isolate 380 under submerged culture supplemented with carboxymethyl cellulose (CMC), microcrystalline cellulose (MMC), rice husk and wheat straw, at 50°C of incubation.

Time of Incubation	CMC	MCC	Rice husk	Wheat straw
2h	0.04±0.004	0.006±0.015	0.04±0.021	0.04±0.021
4h	0.06±0.141	0.04±0.017	0.04±0.021	0.02±0.018
6h	0.12±0.051	0.008±0.020	0.02±0.011	0.02±0.014
8h	0.04±0.0141	0.003±0.0134	0.02±0.013	0.06±0.018
10h	0.08±0113	0.003±0.010	0.06±0.014	0.06±0.014
12h	0.06±0.042	0.016±0.0171	0.06±0.008	0.06±0.015

hours of incubation. Isolate 380 showed a higher performance than the other isolates during the 24 hours of the assay; the best results were obtained at 6 hours of cultivation (Table II).

Assays in submerged culture were performed with isolate 380 to test different cellulose sources: carboxymethyl cellulose, microcrystalline cellulose (MCC), rice husk and wheat straw (Table III), at an incubation temperature of 50°C. The CMC showed the best induction of enzyme production compared to the other substrates. Isolate 380 showed a higher activity at 6 hours of incubation under CMC substrate, performing an enzyme production of 0.12 UEA mL^{-1} min^{-1} (0.02 mg glucose mL^{-1} min^{-1}), followed by wheat straw and rice husk, with the production of 0.06 UEA mL^{-1} min^{-1} (0.01 mg glucose mL^{-1} min^{-1}) at 8 and 10 hours of incubation, respectively. The microcrystalline cellulose substrate provided the lowest enzyme performance (0.04 UEA mL^{-1} min^{-1}).

The precipitation of the CMC culture supernatant by ammonium sulfate at 60% and the enzyme purification by gel filtration chromatography allowed the capture of 20 fractions. Out of that, three fractions showed the highest enzyme activity and were submitted to further assays.

A temperature range from 30 to 80ºC was used to evaluate the thermic stability of the crude extract and the purified enzyme (Fig. 1). Enzyme activity was high at temperature above 50°C for both samples, and a significant activity occurred to 60°C, with a result of 0.14 UAE mL^{-1} min^{-1} activity for crude extract and 0.12 UEA mL^{-1} min^{-1} for purified enzyme. These results confirm the thermophilic enzyme activity profile of the cellulolytic isolate 380.

Enzyme activity evaluation of the crude extract, at pH values ranging from 4.0 to 10.0, was performed. After 30 min, the activity was determined and compared to the result obtained

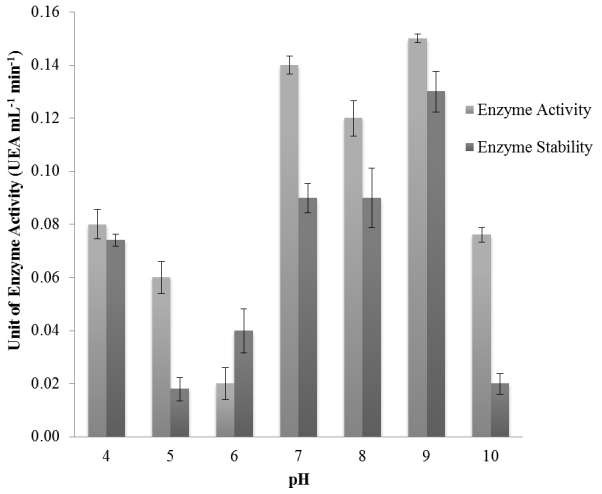

Figure 1 - Evaluation of crude extract and purified CMCase activity face to a temperature range of 30-80ºC after 30 minutes of incubation.

previously by exposition of the crude extract to pH variation. Highest activity was measured at pH 7.0-9.0 (Fig. 2), and more than 60% of the enzyme activity was kept at higher pH range. Furthermore, at pH 9.0, 87% of enzyme activity was kept after the period of incubation tested.

Isolate 380 showed a single band in a SDS-PAGE with a molecular weight of approximately 20 kDa for both crude extract and purified enzyme (Fig. 3). This isolate was a Gram-positive, aerobic, endospore-producing bacterium. The multiple alignments of the nucleotide 16S rDNA sequence (nucleotide length 1,515) by ClustalW showed a high identity (99.7%) with other *Bacillus licheniformis* sequences from NCBI (Fig. 4).

DISCUSSION

Isolate 380 could be identified as *Bacillus licheniformis* (Fig. 4). It was grouped in a clade (86% bootstrap) with *B. licheniformis* SSCL 10 strain identified as a high chitinase producer (2.4 U mL^{-1}) (Abirami et al. 2016) and four other published *B. licheniformis* 16SrDNA sequences from GenBank. Various species of *Bacillus* presented relevant results related to cellulase production (Christakopoulos et al. 1999, Shabeb et al. 2010, Kim et al. 2012, Ladeira et al. 2015, Gaur and Tiwari 2015). In this study, we observed hydrolysis zones of up to 60 mm when the isolate was cultured at 50°C and above 45 mm at 37°C. Moreover, isolates from the compost showed cellulolytic potential at 50°C, with halos ranging from 19 to 21 mm (Chang et al. 2009). Similar results were achieved with our isolates, showing a promising potential for cellulase production.

Carboxymethyl cellulose is a soluble substrate and easily hydrolyzed. In this work, isolate 380 showed an enzyme activity of 0.12 UEA after 6 hours of incubation. A study involving the influence of temperature on enzyme activity showed variations in the production, with 0.001 mg mL^{-1} of the enzyme at 50°C for a production of 344 mg mL^{-1} at 45°C (Shabeb et al. 2010). This result leads us to infer that isolate 380 may show an excellent production of cellulase at low and high temperatures, a crucial factor for its inoculation along the composting process, degrading cellulose sources in mesophilic and thermophilic phases. Furthermore, the hydrolysis of microcrystalline cellulose, for example, requires the synergistic action of different cellulases, which leads to the production of various enzymes.

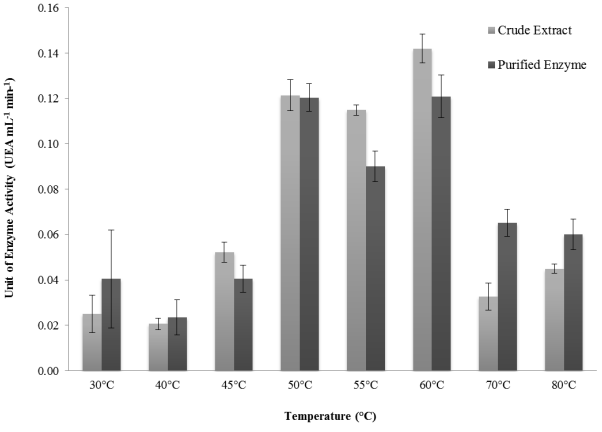

Figure 2 - Evaluation of activity and stability for CMCase crude extract face to pH range of 4.0-10.0. Assay performed after 30 minutes of incubation at 60°C.

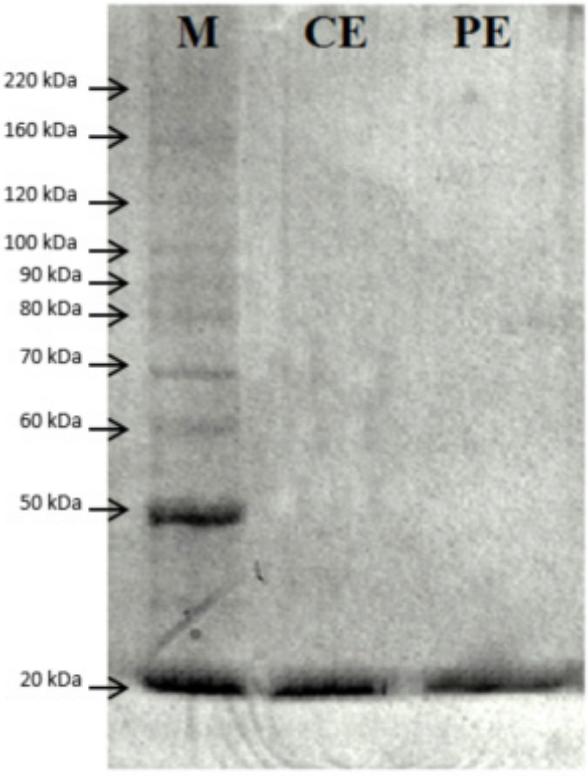

Figure 3 - The molecular weight of crude extract and purified CMCase in SDS-PAGE gel. Staining with Commasie Blue. M: Marker of 20-220 kDa range. CE- crude extract; PE- purified enzyme.

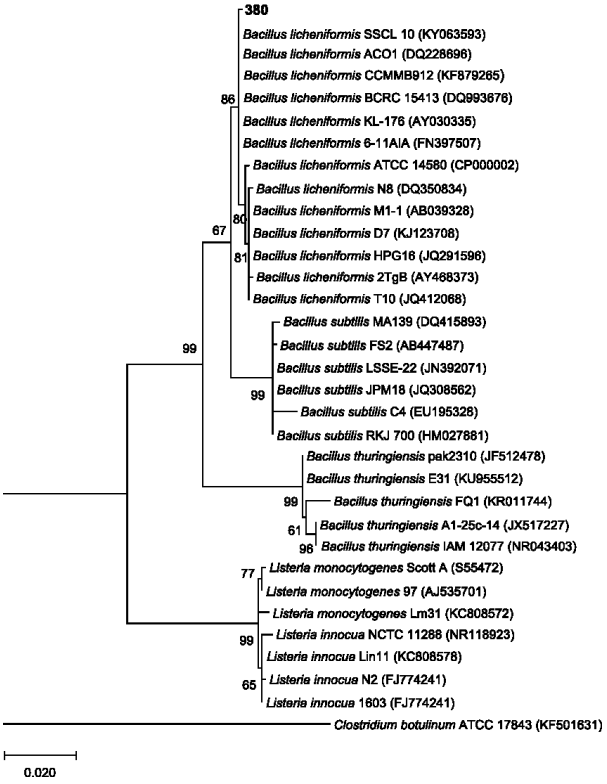

Figure 4 - Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences. The studied isolate is shown in bold. Bootstrap with 1000 resamplings was performed and values greater than 50% shown in nodes.

Also, its crystallinity has a significant influence on this process. Rice husk and wheat straw are lignified and more recalcitrant substrates, and the use of chemical treatments not always improves hydrolysis. Fungi usually hydrolyze wheat straw; however, to achieve complete hydrolysis, heat and mechanical treatments can be used (Gessesse and Mamo 1999, Gyalai-Korpos et al. 2010).

A few studies report the production of cellulases by the genus *Bacillus* under microcrystalline cellulose (Kim and Kim 1995, Waeonukul and Ratanakhanokchai 2007). Its hydrolysis needs the synergistic activity of different cellulases, such as endoglucanases and exoglucanases (Bhat and Bhat 1997, Lynd et al. 2002). Thus, the low activity for microcrystalline cellulose substrates may be linked to an impeded enzyme access to the substrate due to the high degree of crystallinity. The interaction between different cellulases may promote higher hydrolase rates, which explains the synergistic between them (Kim and Kim 1992). The results show that the substrate carboxymethyl cellulose is more suitable for enzyme production under laboratory conditions.

The quantification of total proteins was performed in each step for purification, according to Bradford (1976). Precipitated crude extract and purified enzymes were evaluated. Our results for crude extract and purified enzyme activity (0.14 UEA mL^{-1} min^{-1}) were similar to the findings of Chang et al. (2009) for isolates of the genus *Bacillus.* Rastogi et al. (2010) obtained 0.12 UEA mL^{-1} for crude extract CMCase for *Bacillus* sp. isolated from compost. Previous studies on purified enzyme stability at different temperature ranges have shown similar results (Bischoff et al. 2006, Li

et al. 2008, Korpole et al. 2011). Previous studies observed enzyme activities ranging from 0.1 mg mL^{-1} (Heck et al. 2002) to 6,000 mg mL^{-1} (Yin et al. 2010).

The crude extract assays in this work revealed good stability and enzyme activity (87%) within a pH range from 7.0 to 9.0. Endoglucanases were described for the genus *Bacillus* with activity at pH values from 8.5 to 10.0 (Hakamada et al. 1997, Endo et al. 2001, Aygan et al. 2011). The CMCase activity response of 1.7 UEA mL^{-1} at 60°C and pH 8.0 was obtained by Singh et al. (2004). Several studies report that enzyme activity remains at 100% after a few hours of incubation, but increasing activity by exposure to high temperatures for a few hours is rarely reported; usually, there is a tendency to decreased enzyme activity with longer exposure. Similar to our work, Christakopoulos et al. (1999) evaluated purified endoglucanase from *Bacillus pumilus*, showing 90% of enzyme activity at 60°C. The enzymes undergo the same structural effects as globular proteins in terms of temperature and pH variation. Extreme pH oscillation may change the enzymatic structure due to charge repulsion, while mild changes may induce enzyme dissociation, sometimes leading to more active species or to complete inactivation (Lehninger et al. 2004).

The SDS-PAGE revealed that the 20 kDa bands resulted of crude extract and partially purified enzyme assays for isolate 380. The molecular weight of purified and partially purified cellulases varies largely, from 6 to 80 kDa (Kim et al. 2005, 2009, Gaur and Tiwari 2015). Low molecular weight enzymes, formed by one polypeptide chain and with a disulfide bond, and are generally more stable to heat than oligomeric enzymes with high molecular weight (Furigo and Pereira 2001).

Some microorganisms, such as cellulolytic fungi, show high hydrolysis activity at acidic conditions. Bacteria with cellulase activity are of interest in composting processes with high concentrations of lignocellulosic compounds, thereby reducing the time required to reach the maturation phase. In composting processes, the thermophilic phase is characterized by alkalinization due to the volatilization of nitrogen compounds (Heck et al. 2013), which facilitates alkalophilic enzyme activity. Studies with *Bacillus* sp. isolates evaluated the benefits of microbial additives in agricultural fertilizers and edible mushroom production for the genus *Agaricus* sp. (Figueiredo et al. 2013, Ribeiro et al. 2017). Bacterial cellulase activity facilitates the pre-degradation of lignocellulosic sources, favoring the colonization by secondary decomposers and thus improving substrate use and economic viability of mushroom culture (Dias 2010, Figueiredo et al. 2013).

Enzymes produced by thermophilic bacteria have attracted attention of industrial segment devices due to their high activity and stability at high temperatures, mainly using agro-industrial wastes as substrates for *Bacillus* sp. cellulase producers (Heck et al. 2002, Shabeb et al. 2010). In the composting process, lignocellulosic and recalcitrant compounds are first hydrolyzed by fungi and actinobacteria (Gyalai-Korpos et al. 2010, Pierre et al. 2011). Some studies on industrial cellulase production by *Bacillus* strains are currently being developed due to the high bacterial growth, compared to fungi, and the ability to adapt to low-cost carbon sources, such as sugar cane bagasse. Moreover, bacterial cellulase is a potent enzyme for the application in second generation ethanol produced from sugarcane biomass in Brazil (Acharya and Chaudhary 2012, Ladeira et al. 2015).

CONCLUSIONS

Bacillus licheniformis 380, isolated during composting, was capable of producing alkalophilic CMCase at a relatively high temperature (50°C) in the presence of different substrates. These cellulases may have potential application in biotechnology

industries, as well as bioaugmentation processes, accelerating the degradation of organic matter, especially in edible mushroom culture, degrading cellulosic wastes during primary degradation phase. Further studies evaluating adequate implementation are needed.

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