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Production of Cellulases from a Novel Thermophilic Streptomyces thermocerradoensis I3 Using Agricultural Waste Residue as Substrate

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Abstract

Cellulases and hemicellulases are key enzymes in the utilization of lignocellulosic biomass, an abundant renewable source. In this work, cellulase production by a new thermophilic *Streptomyces thermocerradoensis* was analyzed by cultivation on medium containing carboximethyl cellulose, sugarcane bagasse or wheat bran as carbon source. The best results were obtained with wheat bran. The cellulolytic activity was evaluated by determination of Avicelase, CM Case and FPase activities. Cellulolytic enzymes were characterized by determining the effect of pH and temperature, thermo stability and effects of different metal ions on activities. The pH and temperature profile showed optimal activity at pH 7.0/35° C for Avicelase, pH 4.5/75° C for CMCase and pH 5.0/45° C for FPase. Zymogram analysis showed the presence of multiple cellulases (45, 19 and 17 kDa). The three cellulolytic activities evaluated maintained over 50% of initial activity, even after 4 hours of incubation at 60° C. Cellulases studied in this work are thermophilic, thermos table and active in a wide pH range. They have potential to be used in the development of new biotechnological processes.

Keywords: CMCase, Avicelase, FPase, Thermostability, Wheat bran

1. Introduction

The biomass is an important renewable resource, since it can be converted into various types of raw materials. Cellulose and hemicellulose are the most important polysaccharide of plant cell wall (Gusakov, 2011; Sachez, 2009; Merino and Cherry 2007). Only a small amount of the cellulose, hemicellulose and lignin produced as by-products in agriculture or forestry is used, the rest being considered waste (Sachez, 2009). Cellulose hydrolysis requires synergistic actions of different cellulases. The cellulase enzyme complex consists of three major components: endoglucanase (EG, EC 3.2.1.4), cellobiohydrolase (CBH, EC 3.2.1.91), and β-glucosidase (BG, EC 3.2.1.21). EG acts in random fashion, cleaving β-linked bonds within the cellulose molecule; CBH removes cellobiose units from nonreducing ends of the cellulose chain, and BG degrades cellobiose and cellooligosaccharides to glucose (Tao *et al*., 2010). Similarly, hemicellulose is degraded by different xylanolytic enzymes. Xylan is the main carbohydrate found in hemicellulose. Its complete degradation requires the cooperative action of endo-1,4-β-xylanase (EC 3.2.1.8) and xylan 1,4-β-xylosidase (EC 3.2.1.37) (Sachez, 2009). The conversion of biomass in other chemicals and biofuels has as crucial step the hydrolysis of cellulose into glucose (Tanaka *et al*., 2009).

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The search for an efficient cellulase complex has attracted the interest of many research projects in biotechnology (Nascimento *et al*., 2009; Hideno *et al*., 2011; Soccol *et al*., 2010) . The increase of biomass utilization for bioethanol production may be achieved by decreasing in the cost of enzymes, optimization in the method of pretreatment and improving in fermentation (Olofsson *et al*., 2008). The search of new microorganisms, coupled with studies to increase production of more efficient enzymes, is one of the most effective ways to achieve enlargements in bioethanol production. Another possibility to improve this process is utilization of thermostable enzymes. These enzymes allow the conversion of biomass into biofuel at high temperatures, enabling improvements in the hydrolysis of lignocellulosic substrates, avoiding the risk of potential contamination, increasing the flexibility with regard to the design process, and potentially reducing the reaction time and the enzyme load, improving the overall economics of the process (Menon *et al*., 2010; Rastogi *et al*., 2009). Fungi and bacteria are known as most important producers of cellulases. These microorganisms are commonly found in soils. Tropical soils of Brazil are usually subjected to high temperatures during most of the year. The microbial biodiversity of Cerrado biome is virtually unknown and its potential is little explored. A few studies describe the isolation of new bacterial species from Cerrado soil (Barros *et al*., 2003; Quirino *et al*., 2009). Some research demonstrated that agricultural activities modify the microbial community of the soil, leading to an increase of Actinobacteria (Quirino *et al*., 2009). Actinomycetes are a group of Gram-positive and filamentous bacteria, biotechnologically important due its ability to produce several enzymes and secondary metabolites. The genus Streptomyces is the most important of this group. Streptomyces produces several enzymes, which works effectively in the hydrolysis of organic compounds presents in soil, including degradation of cellulose, hemicellulose and lignin (Nascimento *et al*., 2009; Vinha *et al*., 2011). The aim of this work was the characterization of cellulases produced by thermophilic *Streptomyces thermocerradoensis* I3, an efficient microorganism in degradation of lignocellulosic materials.

2. Material and Methods

2.1 Cellulase Production Using Different Lignocellulolytic Wastes

The *S. thermocerradoensis* I3 was cultivated on minimal medium, supplemented with 0.1% yeast extract (YE) and 0.5% carboxymethyl cellulose (CMC) or 0.5% sugarcane bagasse (SCB) or 0.5% wheat bran (WB). Cells were cultivated at 45 °C for 144 h with constant agitation (120 rpm). After incubation, the culture was centrifuged and the supernatant was used as enzyme source. Samples were analyzed for the activity of CMCase (Lima *et al*., 2005; Ghose, 1987).

2.2 Determination of Optimal Time for Cellulase Production and Activity

Spore suspension (10^6 spores) of *S. thermocerradoensis* I3 was inoculated into 125 mL Erlenmeyer flask containing 50 mL of minimal medium, supplemented with in 0.1% YE and 0.5% WB and incubated for 12 days at 45 °C with constant agitation (120 rpm). Samples were daily collected, filtrated and centrifuged. Protein and cellulase activities were measured in supernatants. Results were presented as an average of three replicates.

2.3 Enzymatic Assays

CMCase activity was assayed by measuring the release of reducing sugars (RS) in a reaction mixture of 1.0 mL of the enzyme and 1.0 mL of 2% (w/v) CMC solution in 50mM sodium citrate buffer (pH 4.8) incubated at 50 °C, for a period of 10 min. RS were measured by DNS method (Miller, 1959). FPase activity was assayed by measuring the release of RS in a reaction mixture containing Whatman No. 1 filter paper (1.0 cm×6.0 cm) as substrate in 50 mM sodium citrate buffer (pH 4.8) at 50 °C, after 30 min. Avicelase activity was measured by measuring the release of RS in a reaction mixture of 250 µL of the enzyme and 500 µL of 1% (w/v) avicel solution in 50 mM sodium citrate buffer (pH 4.8) incubated at 50 °C, for a period of 60 min. One enzyme unit (U) corresponded to 1 µmol of glucose equivalents released per minute under the assay conditions (Ghose, 1987).

2.4 Determination of Optimal Temperature and Ph, Thermo stability, and Influence of Metal Ions

The optimal temperature was determined by performing enzymatic assays in temperature range 20 to 100 °C at pH 4.8. The optimal pH determination was carried out using 50 mM buffers solutions ranging from pH 3.0 to 10.0. The following buffers were used: sodium citrate for pH 3.0–6.0, sodium phosphate for pH 6.0–8.0, Tris–HCl for pH 9.0, and glycine–NaOH for pH 10.0. To study the thermo stability, samples were pre-incubated at 50 °C and 60 °C for 0.5, 1, 2, 3, 4, 6 and 8 h.

The influence of metal ions on the activities was performed in each optimal conditions and adding tests ions (aluminum, barium, calcium, potassium, magnesium, sodium, manganese and ammonia in the chloride form) at 10 mM final concentration. The influence of EDTA was also analyzed. All experiments were performed in triplicate and the results expressed as mean values (Lima et al., 2005; Brito-Cunha et al., 2013).

2.5 Zymogram Analysis

For zymogram analysis, sample was precipitated with cold acetone (1:2) and analyzed in 10% SDS-PAGE, containing 0.2% CMC (w/v). Sample containing 0.5 U was loaded. After SDS-PAGE, the gels were incubated for 1 hour in 1% Triton X-100 at room temperature, followed by incubation for 12 hours at 50 °C in 50 mM sodium citrate buffer (pH 4.8) and stained with 0.1% Congo red for 20 min. The gels were washed with 1 M NaCl until visualization of bands of the enzymes (Blum *et al*., 1999).

2.6. Saccharification Assays

The saccharification assays were performed using SCB pretreated by steam explosion (Kovacs *et al*., 2009). The hydrolysis reactions were carried out in 125 mL Erlenmeyer flasks in a reciprocal shaker at an agitation rate of 120 rpm for 48 hours at 50 °C in sodium citrate buffer 50 mM and pH 5 at a final volume of 10 mL (Hsu *et al*., 2011). The enzymatic hydrolysis reactions were carried out using culture supernatant of the 6th and 12th day of incubations. The reactions were supplemented with β-glucosidase from *Aspergillus niger*. Table 2 shows the composition of hydrolysis reactions. The reaction of saccharification was carried out at two conditions: R I at 50 °C for 48 hours; R II at 70 °C for initial 2 hours and 50 °C for 46 hours. The R II has two modifications relative to R I. 1 - only the enzyme produced in 6th was added. 2 - the reaction was initially incubated at 70 °C. After two hours, enzyme produced in 12th day and β-glucosidase was added and the reaction was incubated at 50 °C. Those modifications were introduced because CMCase showed highest activity at 70 °C. After this period, the hydrolysis reactions were filtered through a filter paper. The concentration of RS (DNS method) and glucose (glucose oxidase Reagent kit-doles ® following the manufacturer's guidelines) were determined. Xylose concentration was obtained by subtracting glucose from the total RS (Kovacs, 2009).

2.7. Thin-layer Chromatography (TLC)

Thin layer chromatography was performed on silica gel plate (DC-Fertigfolien Alugram SIL G/UV254 Xtra ®). A total of 2.5 µL of glucose (1%), cellobiose (1%) or xylose (1%) were used as standards. Ten microliters of reactions I and II were analyzed to identify the products of hydrolysis, the plate was placed into a mobile phase containing butanol, methanol and water (4:2:1) for reading the plate was sprayed with a solution of revealing containing phosphoric acid 85% (7.5 mL), aniline (1.0 mL), diphenylamine (1.0 g) and acetone (50.0 mL), and subsequently the plate was incubated at 100 °C until complete visualization of the bands (Pietrobon *et al*., 2011) .

3. Results and Discussion

In previous work we have isolated and studied a new thermophilic *Streptomyces sp*., we have studied the characterization of xylanase produced by *S. thermocerradoensis* I3 grown in presence of different lignocellulosic materials (Brito-Cunha *et al*., 2013). During the realization of xylanase characterization, it was noted that *S. thermocerradoensis* I3 also produced cellulases. In light of this observation, the characterization of cellulolytic enzymes has become the object of our interest.

3.1 Effect of Carbon Source on the Cellulase Production

The *S. thermocerradoensis* I3 was inoculated in minimal medium broth supplemented with: CMC, SCB or WB as carbon source. The ability to produce cellulase was measured by determination of the CMCase activity. The highest CMCase activity (2.052 U/mL) was obtained when the microorganisms was grown in medium supplemented with WB (Fig. 1). Several carbon sources have been used in researches involving the production of cellulases. WB has been used as carbon source in some works. Some studies found that the higher production of cellulase was achieved when WB was used as carbon source, what is in accordance with results obtained in present work (Nascimento *et al*., 2009; Vinha *et al*., 2011; Lima *et al*., 2005; Goldbeck *et al*., 2013) The cellulases production by *S. viridobrunneus* SCPE-09 grown in various sources of carbon was evaluated. The highest yield was obtained when grown in WB (Vinha *et al*., 2011). Another study showed that the highest production was found when *S. drozdowiczii* grown in the presence of CMC. In the presence of WB only 60% of higher production was obtained (Lima et al., 2005). WB is a good source of carbohydrates and proteins. WB contains 34% starch, 18% xylan, 10.5% glucan, 10.1% arabinan, 1.1% galactan and 5% lignin. Besides these polysaccharides, it still has 13.5% protein.

This composition of polysaccharides (both qualitatively and quantitatively) and proteins, probably exert a positive influence on enzyme production by this microorganism, resulting in a higher enzyme production in the presence of WB, when compared to other sources (Sun *et al*., 2008).

3.2 Kinetic of Cellulases Production

In order to evaluate the effect of incubation time on cellulases production, *S. thermocerradoensis* I3 was cultivated during 12 days and the enzymatic activity of cellulases present in the supernatant was evaluated daily. The cellulolytic activity was estimated by determination of Avicelase, CMCase and FPase activities. The highest Avicelase production was observed on the eleventh day (5.646 U/mL), however on the second day another peak of activity (2.62 U/mL) was observed. CMCase (3.872 U/mL) and FPase (0.0947 U/mL) showed higher activities on the sixth day (Fig. 2).

3.3 Effect of Temperature and pH on Cellulolytic Activity

The influence of pH on cellulolytic activity was investigated in the pH range 3.0 to 10.0. As can be seen in Fig. 3, the activities of Avicelase, CMCase and FPase exhibit different behaviors. Avicelase showed maximum activity at pH 7.0 and maintained activity in a pH range from 4.0 to 10.0 (around 60% of maximum activity was retained). CMCase showed the highest activity at pH 5.0 and showed a narrower range, varying between 3.5 and 7.5. Analyzing the profiles of FPase, two different peaks were observed (Fig. 3). This fact suggests the existence of at least two enzymes with different optimal pH (pH 6.0 and pH 8.0). The higher FPase activity was observed at pH 8.0. The results obtained were in accordance with results of others studies about cellulase produced by Streptomyces. Many CMCases show optimal activity between pH 4.0 to 6.0: *S. malaysiensis* at pH 5.0 (Nascimento *et al*., 2009), *Streptomyces sp.* at pH 5.0 (Alani *et al*., 2008), *S. ruber* at pH 6.0 (El-Serry *et al*., 2010), *S. drozdowiczii* at pH 5.0 (Lima *et al*., 2005) and *S. viridobrunneus* SCPE-09 at pH 4.9 (Vinha et al., 2011). There are also cellulases produced by *Streptomyces sp*. that are effective in neutral and alkaline pH. The cellulolytic extract produced by two different *Streptomyces sp.* isolated from soil showed maximum CMCase activity at pH 7.0 and 7.5 (Chellapandi and Himanshu, 2008). Two peaks of CMCase (pH 7.0 and pH 11.0) were observed in study about cellulase production by *Streptomyces sp.* (Semedo *et al*., 2000). The optimum pH for Avicelase activity produced by *S. reticuli* was pH 4.5-5.0 (Walter and Schrempf, 1996), while for Avicelase activity produced by *Streptomyces sp* was obtained at pH 7.0 (Tuncer *et al*., 2004).

Studies about FPase produced by Streptomyces are rare. The pH profile of FPase produced by two strains of *Streptomyces sp.* (M7a and M23) was studied. Two peaks of activity were observed at pH 5.6 and 9.0 in both strains. Theirs activities were 1.43 and 1.78 U/L for strain M7a and 1.43 U/L and 1.78 U/L for strain M23 (Semedo *et al*., 2000). The results described for FPase in this work, two peaks of FPase at pH 6.0 (3.5U/mL) and 8.0 (3.8 U/mL) (Fig. 3), are consistent with these authors (Semedo *et al*., 2000). But the production of FPase by *S. thermocerradoensis* I3 studied in this work is higher. The temperature profile presented by cellulases analyzed is quite intriguing. The extract appears to contain different cellulolytic enzymes, since activity peaks were visualized at different temperatures. Three peaks of CMCase activity were observed (35, 50 and 70 °C). Two peaks of Avicelase (35 and 50 °C) and only one for FPase (45 °C). The highest CMCase activity was obtained at 70 °C, indicating that this celullase can be considered as thermophilic (Fig. 4). The analysis of pH and temperature evidenced for cellulases activity suggested the presence of multiple cellulases produced by *S. thermocerradoensis* I3. The production of several cellulases is common in nature, especially in actinomycetes (Vinha *et al*., 2011; Brito-Cunha *et al*., 2013; Alani *et al*., 2008; George *et al*., 2001; Nascimento *et al*., 2003). Production of multiple cellulases and hemicellulases by microorganisms improved the action and the synergism of enzymes, resulting in a better degradation of lignocellulosic materials.

3.4 Thermostability Analysis

The thermostability of the cellulolytic enzymes presented in the culture supernatant was studied by incubation at 50 °C and 60 °C for at least 8 hours. The three cellulolytic activities evaluated maintained over 50% of initial activity after 4 hours of incubation at 50 °C and 60 °C (Fig. 5). Approximately 40% of the activity was retained even after 8 hours of incubation. The incubation at 50 °C for at least 3 hours had a positive effect on the activity of CMCase, since residual activity is higher than initial activity. It can be assumed that cellulolytic activities can be classified as thermostable, since more than 50% of initial activity was retained after 4 hours of incubation at 60 °C.

The CMCase of *S. drozdowiczii* showed maximal activity at 50 °C, but less than 35% of activity was retained after 2 hours of incubation at 50 °C (Lima *et al*., 2005). CMCase produced by *S. malaysiensis* showed higher activity at 60 °C, however residual activity showed less than 10% of initial activity after 30 minutes at 60 °C (Nascimento *et al*., 2009).

3.5 Effect of Metal Ions on Cellulases Activity

Studies concerning metal ions influence are very important for industrial enzyme applications. Metal ions may be required for enzyme activity, as part of the enzyme complex or serve as cofactors to achieve maximal activity. Manganese and other metal ions can increase the affinity of the enzyme to the substrate and/or may stabilize the conformation of the catalytic site (Chauvaux et al., 1995). Cellulases are generally inhibited by Fe^{2+} and Cu^{2+}, however Mn^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} are not inhibitors, although they have similar sizes and loads (Tejirian and Xu, 2010). Avicelase noticed a significant inactivation of 78% for ion Mg^{+2}, 55.3% for Mn^{+2} and 51.7% for Al^{+3}. For the CMCase activity we founded a significant inactivation of 54.4% with EDTA. For the activity FPase noticed a decrease of 41.3% of the activity in the presence of ion NH_4^+, the other ions do not showed significant result. The ion Cu^{+2} causes an inhibition of 30%, while the K^+ and Ba^{+2} increased the CMCase activity from *S. drozdowiczii* by 62 and 85%, respectively (Lima *et al*., 2005). Considerable decrease in activity in the presence of Cu^{+2}, Fe^{+2}, and Mn^{+2} was observed in CMCase activity from *S. viridobrunneus* SCPE-09 (Vinha *et al*, 2011). Some ions are cited in literature as inhibitors for microbial cellulase. Activity is probably inhibited through the attack of certain groups at the active site of the enzyme resulting in the inactivation (Chauvaux et al., 1995; Tejirian and Xu, 2010). Inhibition also can occur by binding of ions to the thiol group, which is part of the active site of such enzymes. The authors showed that the use of DTT (dithiothreitol) may increase the action of cellulases by maintaining the sulfhydryl group at the active site in its reduced form (Jonhson and Damain, 1984).

3.6 Zymogram Analysis

To perform the zymogram, the culture supernatant of the sixth day of growth was precipitated with cold acetone. Zymogram analysis showed three bands (45, 19 and 17 kDa) with activity after SDS-PAGE and Congo red staining (Fig. 6). The obtained results corroborate the temperature profile suggesting the existence of multiple cellulases in this extract, when these isolate growing in the presence of WB as carbon source. In the genus Streptomyces the presence of multiple cellulases is relatively common. Zymogram analysis of culture supernatants from *S. viridobrunneus* SCPE-09 showed two bands on the fifth day of fermentation (apparent molecular masses of 37 and 119 kDa). However, the temperature profile showed a single peak (50 °C) (Vinha *et al*., 2011). Likewise, three cellulolytic bands were observed in a study with *S. malaysiensis* (Nascimento *et al*., 2009). A system of multiple cellulases containing 5 isozymes produced by *S. antibioticus* was described (Enger and Sleeper, 1965). Other bacteria also have a system of multiple cellulolytic enzymes, *Bacillus licheniformis* showed the presence of 5 bands in zymogram analysis (Bischoff et al., 2006).

3.7. Saccharification Assay and TLC Analysis of Reaction end Products

The hydrolysis of lignocellulose into monosaccharaides is the bottleneck in the conversion of biomass into chemicals and biofuels. The saccharification needs the action of cellulases and xylanases complexes. The synergistic action of those enzymes allows the release of glucose and xylose. The higher production of CMCase occurs at the 6th day, while the best xylanase production occurs at 12th (Brito-Cunha *et al*., 2013). Aiming to obtain higher yield, saccharification assay was carried out with combination of enzymes produced in this two day of incubation. The observed results are reported in Table 2. The procedure II was the most efficient in releasing of monosaccharaides. In R II the rate of conversion to glucose was 19% (0.86 g/L) and to xylose was 62.9% (2.14 g/L), while in R I the rate of conversion was 16% for glucose (0.72 g/L) and 57 % for xylose (1.93 g/L). The R II showed an increase of 20% in glucose conversion and 10% xylose conversion when compared to R I. The Increase in temperature in the initial two hours probably enhanced the efficiency of cellulases, principally the CMCase activity, resulting in greater exposure of pulp fiber, allowing action of others enzymes present in the crude extracts. The products of saccharification were analyzed by TLC. Fig. 7 shows the formation of monosaccharaides in both reactions, with higher production in R II. Further studies will be necessary for the optimization of reaction conditions, which may improve saccharification rate, releasing more glucose and xylose. Corn cob hydrolyzate was digested with CMCase, Avicelase and β-glucosidase produced by *Streptomyces sp.* T3-1. The synergistic interaction of endoglucanase, exoglucanase and β-glucosidase resulted in an efficient hydrolysis. After 5 days of incubation, 53.1% of biomass was converted in monosaccharaides (Hsu *et al*., 2011). The enzymatic hydrolysis of SCB pretreated with acid was evaluated.

Four commercial enzymes were used. The results showed that pretreatment using acid and enzyme CL gave the best result, releasing about 45% of glucose (Pietrobon *et al*., 2011). Corn cob pretreated with NaOH was efficiently hydrolyzed by commercial enzymes, reaching 27% yield in glucose release (Yoon *et al*., 2006). The results obtained in this work, although release less monosaccharaides, show the potential of biotechnological utilization of this crude enzymes, once studies of stabilization or optimization of their activity can improve their efficiency.

4. Conclusions

Due to lack of robust enzymes that act efficiently at elevated temperatures and are effective over a wide pH range, some bottlenecks are observed in the hydrolysis of lignocellulosic biomass. The search for new cellulolytic bacteria that produce cellulases displaying these characteristics has aroused great interest. Cellulases studied in this work are thermos table and active in a wide pH range, thus they have potential to be used in biotechnological process for conversion of biomass into chemicals and biofuels.

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Figure 1: Cmcase Production by *Streptomyces sp* (Isolates 3, 7 and 12) Growing on Minimal Medium Supplemented with 0.5% SCB, CMC or WB, at 45 °C For 05 Days under Constant Agitation (120 Rpm)

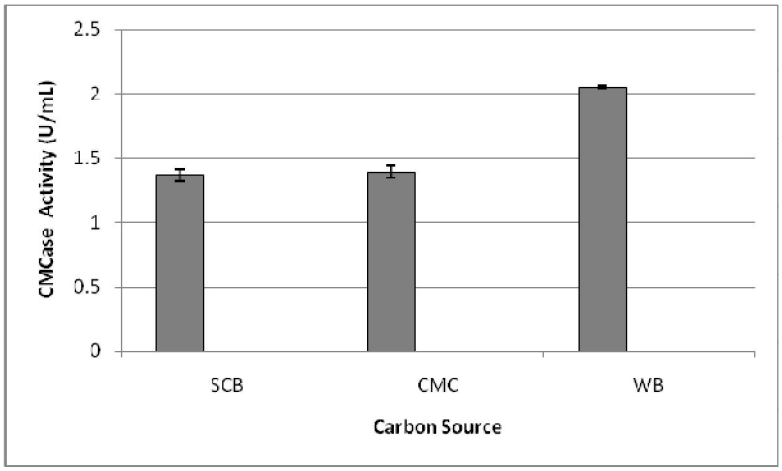

Figure 2: Cellulases Productions by *S. thermocerradoensis* I3 Growing on Minimal Medium Supplemented with 0.5% (W/V) WB, At 45 °C for 12 Days Under Constant Agitation (120 Rpm): Avicelase Activity (Blue), Cmcase Activity (Red), Fpase Activity (Green)

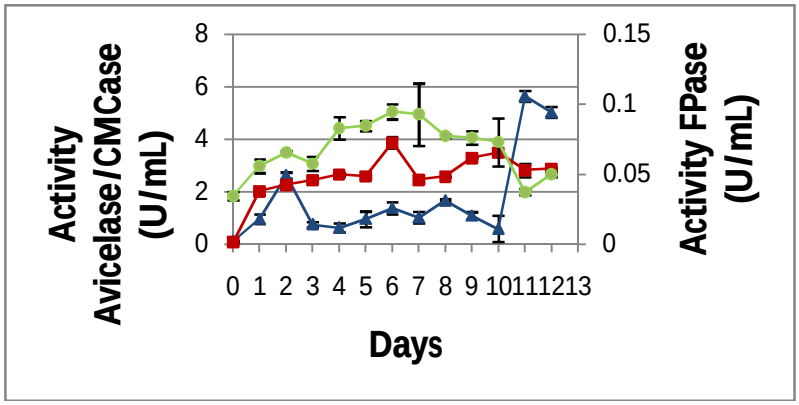

Figure 3: Effect of Ph on Activities (50 °C) of The: Avicelase (Blue), Cmcase (Red) and Fpase (Green) Produced by S. thermocerradoensis I3 Grown on Minimal Medium with 0.5% (W/V) WB. The Ionic Strength for all Buffers was 0.05 Mol/L: Sodium Citrate (Ph 3 to 6), Sodium Phosphate (Ph 7 and 8), Tris-Hcl (Ph 9) and Glycine-Naoh (Ph 10). Residual Activity is Expressed as a Percentage of the Maximum

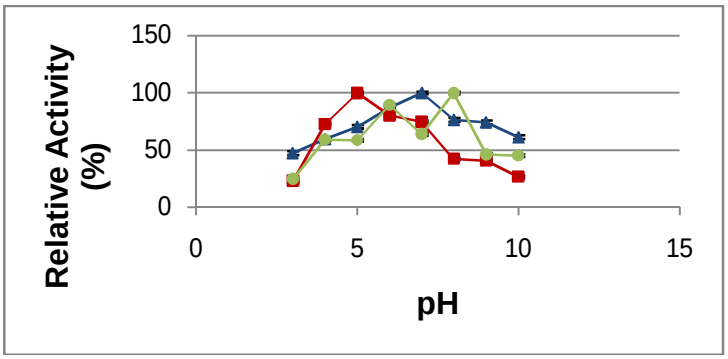

Figure 4: Effect of Temperature on: Avicelase Activity (Blue, Ph 7), Cmcase Activity (Red, Ph 5) and Fpase Activity (Green, Ph 8) Produced by S. thermocerradoensis I3 Grown on Minimal Medium with 0.5% (W/V) WB. Residual Activity is Expressed as a Percentage of the Maximum

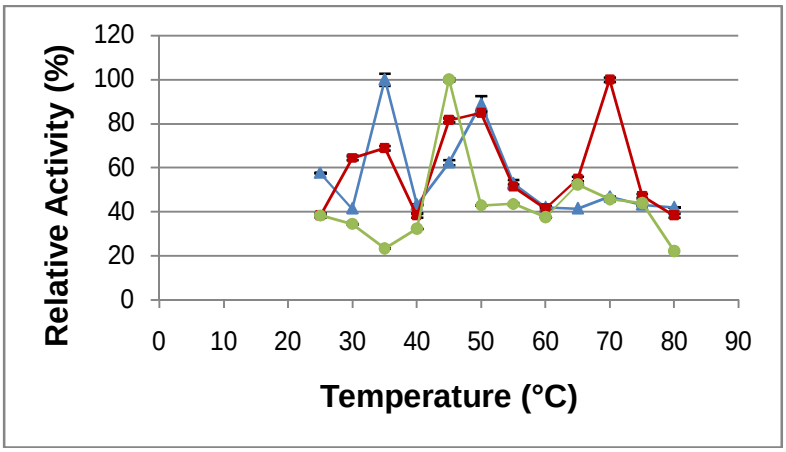

Figure 5: Thermostability of Cellulases Produced by S. thermocerradoensis I3 at Temperatures of 50 °C and 60 °C. Avicelase Activity (Blue), Cmcase Activity (Red) and Fpase Activity (Green). Relative Activity Expressed as Percentage of Original Activity. Solid Line 50 °C, Dashed Line 60 °C.

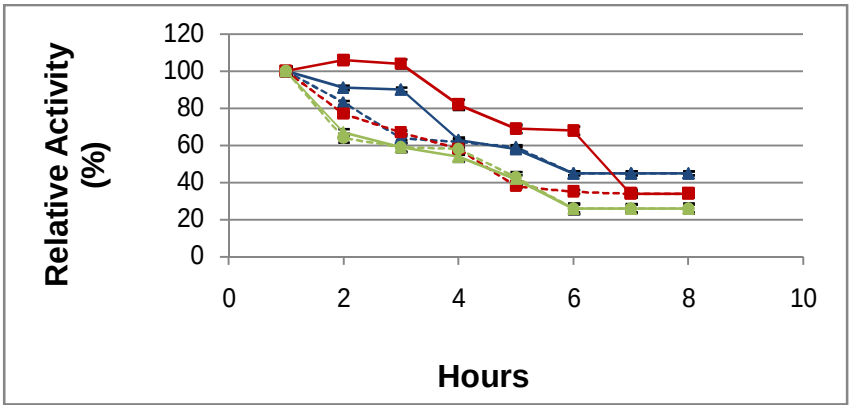

Figure 6: Zymogram Analysis of Cmcase Activity in the Supernatant of S. thermocerradoensis I3 Grown in Presence of 0.5% (W/V) Wheat Bran. The Amounts Enzyme Loaded in the Gel Contained 0.5 U Of Cmcase

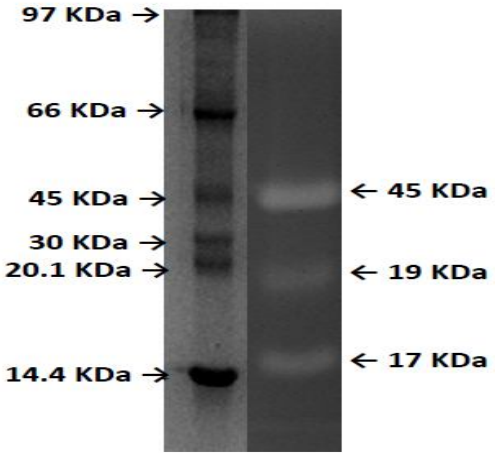

Figure 7: TLC of Saccharification Products of Reactions R I and R II. G (Glucose), C (Cellobiose) and X (Xylose) Were Used as Standard Sugars

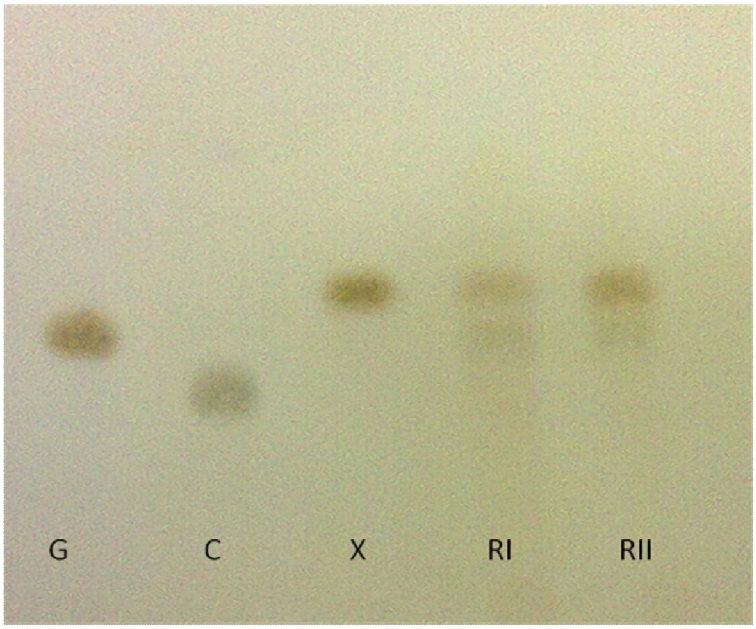

Table 1: Effect of Different ions on Avicelase, CMCase and FPase Activity. The Final Concentration in the Reaction Mixture was 10 mM (all ions are in Chloride form)

	Relative Activity (%)		
Metal ions	Avicelase	CMCase	FPase
Control (no addition)	97.87 ± 1.3	100 ± 1.5	100 ± 0.04
Al^{+3}	46.93 ± 0.6	76.75 ± 1.0	63.49 ± 0.15
Ba^{+2}	76.01 ± 1.0	65.73 ± 0.5	83.56 ± 0.16
Ca^{+2}	62.55 ± 0.9	67.48 ± 2.1	67.61 ± 0.07
K^{+}	79.17 ± 1.0	71.07 ± 0.6	93.75 ± 0,11
Mg^{+2}	22.23 ± 0.3	61.95 ± 1.4	75.95 ± 0.04
Na^{+}	55.70 ± 0.7	74.24 ± 0.3	97.77 ± 0.01
Mn^{+}	47.70 ± 0.6	98.28 ± 1.3	79.96 ± 0.05
NH_4^{-}	81.40 ± 1.1	63.67 ± 0.6	61.02 ± 0.11
EDTA	94.56 ± 1.3	45.66 ± 0.5	80.78 ± 0.06

Table 2: Incubation Conditions and Composition in the Saccharification Analysis

Enzyme	6th day	12th day	Total (U/mL)	RI		RII	
				Glucose	Xylose	Glucose	Xylose
Avicelase	0.048	0.152	0.200	0.72 g/L	1.93 g/L	0.86 g/L	2.14 g/L
CMCase	1.580	1.160	2.740				
FPase	0.260	0.230	0.490	16.0%	19.0%	19.0%	62.9%
Xylanase	16.800	31.200	48.000				
β-glucosidase	-	-	0.021				