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Saccharification of Lignocellulosic Materials by Cellulolytic and Xylanolytic *Paenibacillus illioisensis* CX11

Abeer Ahmed Qaed Ahmed*, Tracey McKay

University of South Africa
South Africa

* *Corresponding author's email: abeer01ahmed01 [AT] yahoo.com*

ABSTRACT—*The utilization of lignocellulosic materials to produce a variety of building blocks (e.g. fermentable sugars) is an interesting alternative approach to meeting the growing demand for high value chemicals. Cellulose and hemicellulose can be hydrolyzed by cellulase and xylanase enzymes into their respective building blocks (hexoses and pentoses), which can later be converted into the targeted compounds. The aim of this study was to test the ability of Paenibacillus illinoisensis CX11 to saccharify different lignocellulosic materials, and to determine its ability to produce cellulolytic and xylanolytic enzymes for possible use in converting lignocellulosic materials into their respective fermentable sugars. The ability of P. illinoisensis CX11 to produce CMCase, xylanase, FPase, and avicelase was tested using SSF of corn stalk. Furthermore, the ability of P. illinoisensis CX11 to saccharify lignocellulosic materials was tested using corn stalk, wheat bran, sawdust, and corn cob. The amount of reducing sugars released from the saccharification of lignocellulosic materials was determined by the 3,5-dinitro-salicylic acid (DNS) method. Obtained results showed that P. illinoisensis CX11 can produce CMCase (400.12 ± 1.23 U/L), xylanase (385.57 ± 2.25 U/L), FPase (266.93 ± 2.22 U/L), avicelase (187.85 ± 2.22 U/L) and extracellular protein (4.56 ± 0.14 mg/L). Moreover, P. illinoisensis CX11 showed an ability to saccharify lignocellulosic materials. These findings confirm that P. illinoisensis CX11 has the ability to produce cellulolytic and xylanolytic enzymes, and to hydrolyze different lignocellulosic materials into fermentable sugars. Therefore, this study concludes that P. illinoisensis CX11 can be considered a good source of cellulase and xylanase enzymes to saccharify different lignocellulosic materials.*

Keywords— Avicelase, FPase, lignocellulosic materials, *Paenibacillus illinoisensis* CX11, saccharification

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1. INTRODUCTION

The production of chemicals has been dominated by petroleum. However, the limitations of and concerns arising from the dependency on fossil resources have emphasized the importance of finding renewable, cost-effective alternative resources [1]. As chemical production is highly dependent on resources, the uncertain status of fossil resources may negatively affect chemical production in the future; therefore it is important to find and develop alternative resources.

It is well known that biomass conversion can produce a tremendous amount of chemicals. In particular, lignocellulosic materials are a highly economical and renewable natural carbon resource, with annual production reaching more than 150 billion tons [2]. Thus, the use of lignocellulosic materials as a renewable source in chemicals production offers the possibility of significant economic rewards. Lignocellulosic materials, which are composed of lignin (25–30%), hemicellulose (25–30%) and cellulose (35– 50%), can be converted into a wide range of chemicals [1,3,4]. Lignocellulosic materials are an important source of energy and chemicals. However, they are not useful in their polymeric forms, and must first be converted into useful smaller molecules (i.e., fermentable sugars and other biochemicals) [5]. Depending on the composition of the components of the lignocellulosic materials, their respective building blocks can be converted into different targeted products.

Cellulose is a high molecular linear homopolymer that consists of D-glucose linked by β-1,4-glycosidic bonds, with basic coupling units of cellobiose [6]. Cellulase enzymes (endoglucanases, cellobiohydrolases, and β-glucosidases) have different modes of action that enable them to work synergically to break down cellulose into the monomeric molecules of glucose [7,8]. As it is estimated that several billion tons of cellulose are produced annually [6], this could constitute a good source of the glucose required for several different industrial processes, as a renewable alternative to petro-based resources.

Hemicellulose is a polysaccharide with a basic chain that consists of residues of D-xylose, D-glucose, D-galactose, D-mannose, and other glycosyls, linked to the basic chain as branched chains [6]. The structure of hemicellulose that consists of C5 and C6 sugars would be hydrolysis by a mixture of enzymes. Xylan hydrolyzed by xylanases through hydrolyzing β-1,4 linkages to produce oligomers that can be further hydrolyzed by β-xylosidase to xylose [8]. Depending on the composition of hemicellulose, other enzymes such as α-L-arabinanases, arabinofuranosidases and β-mannanases are also required in the hydrolyzing processes [8,9]. The different monomers of hemicellulose can be used in the production of chemicals, antibiotics, fuels, and alcohols [10]. Thus, hemicellulose has enormous potential for numerous industrial applications.

Microorganisms can produce a wide variety of enzymes that degrade the rigid and complex structure of lignocellulosic materials [11-13,8]. In recent years, considerable attention has been focused on the enzymatic degradation of cellulose and hemicellulose as an attractive approach to hydrolyzing lignocellulosic materials [14,15]. The fermentation of lignocellulosic sugars has been used to produce highly valued compounds and products [16-20]. This process consists of two major steps, of which the first includes converting the cellulose and hemicellulose in the lignocellulosic materials into their respective fermentable sugars, and the second involves fermenting the released sugars into the targeted compounds.

The fermentable building block sugars from lignocellulosic materials can be converted into a variety of chemicals, such as acetic acid, succinic acid and itaconic acid, all of which can be used in numerous industries, such as the food, chemical and pharmaceutical industries [16-20]. Thus, the full utilization of lignocellulosic materials could put multiple industries that rely on chemicals for production on a path to sustainable development. It is the most promising strategy for the conversion of low value materials (or even waste materials) into useful, high value chemicals and products.

In a previous work, *Paenibacillus illinoisensis* CX11 (accession number LC176650) was isolated and found to have the potential to produce lignocellulolytic enzymes [21]. However, *P. illinoisensis* has not been previously reported to have the ability to hydrolyze different lignocellulosic materials. Thus, the aim of the study reported on here was to test the ability of *P. illinoisensis* CX11 to saccharify different lignocellulosic materials and to produce cellulolytic and xylanolytic enzymes, including FPase and avicelase. This could determine the potential use of *P. illinoisensis* CX11 as an enzymatic source in the first step of converting lignocellulosic materials into fermentable sugars prior to their later conversion into the targeted compounds.

2. MATERIAL AND METHODS

2.1 Chemicals

Media components, chemicals and reagents were purchased from Sigma-Aldrich Pty. Ltd. (Johannesburg, South Africa). All chemicals and reagents were of the best analytical grade available.

2.2 Enzyme extraction using solid state fermentation (SSF)

Enzymes were extracted by inoculating 10 ml of the homogenous suspension of *P. illinoisensis* CX11 into three sterilized flasks (250 ml) containing 10 g of ground corn stalk moistened with 20 ml of distilled water. Flasks were incubated at 30°C for 3 days. On the third day of incubation, 10 ml of sterilized distilled water was added to each flask. The contents were well mixed and further incubated until the sixth day. Enzymes were extracted by adding 100 ml of sterilized distilled water to each flask, and the flask contents were filtered through muslin cloth. Filtrates were centrifuged at 8000 g and 4°C for 15 min. Resultant supernatants were used as crude enzymes for protein determination and enzyme assays.

2.3 Determination of protein

Protein determination was performed by following a previously described method [22]. Culture supernatant (1 ml) was added to a tube containing 0.5 ml of reaction mixture. Distilled water was used as a blank (1 ml). All tubes were left at room temperature for 10 min, and then Folin’s reagent (0.5 ml) was added. The tubes were left at room temperature for 20 min. Finally, the absorbance was measured by spectrophotometer at 720 nm. A bovine serum albumin (BSA) standard curve was used to determine the concentration of protein.

2.4 Enzyme assays

CMCase, xylanase, FPase and avicelase activities were determined using the 3,5-dinitrosalicylic acid (DNS) method [23]. The reaction systems were prepared as follows: 1 ml of the crude enzyme supernatant and 1 ml of CMC (1%) in 0.1 M of sodium acetate buffer (pH 5.0) for determining CMCase activity [24]; 1 ml of culture enzyme supernatant and 1 ml of xylan from birch wood (2%) in sodium acetate buffer (pH 5.5) for xylanase activity determination [25]; 1 ml of crude enzyme supernatant and 2 ml of citrate buffer 0.1 M (pH 4.8) containing 50 mg of Whatman No. 1 filter paper for FPase

activity determination [26]; and 1 ml of crude enzyme supernatant added to 1 ml of avicel 2% (w/v) in 0.1 M of phosphate citrate buffer (pH 6.6) for the determination of avicelase activity [27]. Mixtures were incubated for 30 min at 50°C for CMCase and xylanase activity, 60 min at 50°C for FPase activity and 40°C for 2 h for avicelase activity. After incubation, 3 ml of DNS reagent was added to the mixtures and then incubated in a boiling water bath for color development, after which the absorbance at 540 nm was measured. One unit of enzyme activity (U) was defined as the amount of enzyme that releases 1µmol of product (glucose or xylose) per ml of culture per min.

2.5 Ability of P. illinoisensis CX11 to saccharify different lignocellulosic materials

Corn stalk, wheat bran, sawdust, and corn cob were used to test the ability of *P. illinoisensis* CX11 to saccharify lignocellulosic materials. The lignocellulosic materials were dried, cut and ground in an electric grinder. Five grams of the ground lignocellulosic materials were placed in 250 ml Erlenmeyer flasks and moistened with distilled water (10 ml), and sterilized at 121°C for 15 min. Ten ml of inoculum size 5.0×10^{7} CFU/ml of *P. illinoisensis* CX11 was added to each sterilized flask containing one of the lignocellulosic materials. Inoculated flasks were mixed gently and then were incubated for 3 days at 30°C. On the third day of incubation, 5 ml of sterilized distilled water was added to each culture and mixed well prior to further incubation until the sixth day.

After incubation, 50 ml of distilled water was added to each flask and the flask was shaken for one hour at 200 rpm. Flask contents were filtered through clean muslin cloth in a glass funnel. Filtrates (sugar solution) were subjected to centrifugation at 8000 g for 10 min. All supernatants were further used in the determination of total reducing sugars.

One ml of each sugar solution was put into a glass tube, and then 1 ml of DNS reagent was added to the same tube. A blank was prepared by adding 1 ml of DNS reagent to 1 ml of distilled water. All tubes were incubated at 75°C in a water bath for 10 min and were left to cool down at room temperature. One ml of potassium sodium tartrate was added (40%) to each tube. Total reducing sugars using glucose (TRS-G) and xylose (TRS-X) as standards were determined at 540 nm. The experiment was done in triplicate.

2.6 Statistical analysis

Results were analyzed using Microsoft Excel 2013 (Microsoft Corp., USA). The results were presented as mean ± standard deviation of three experiments.

3. RESULTS AND DISCUSSION

The ability of microorganisms to produce cellulases and xylanases has an important role to play in the first step of converting lignocellulosic materials into fermentable sugars prior to converting them into the targeted compounds. Previous studies reported the ability of some bacterial strains to produce cellulase and xylanase enzymes, and their ability to saccharify lignocellulosic materials [28,15]. For instance, *Bacillus subtilis* was found to produce cellulases that have been used to saccharify wheat straw, bagasse and rice straw [29]. Other bacterial isolates, such as *Bacillus felxus*, were found to produce FPase, CMCase, avicelase and xylanase, and were reported for their ability to saccharify agricultural wastes such as rice straw, bagasse, wheat straw, sawdust, potato peels, wheat bran and corn stover [15]. This suggests that such a strategy using microbial enzymes in the saccharification step of lignocellulosic materials is a good approach. Microorganisms with the ability to produce enzymes such as CMCase (endoglucanase), FPase (total cellulase), avicelase (exoglucanase) and xylanase have the ability to degrade the rigid and complex structure of lignocellulosic materials. The synergistic action of cellulase and xylanase enzymes is required for the bioconversion of cellulose and hemicellulose into fermentable sugars. Endoglucanase act randomly on the insoluble and soluble chains of cellulose, while exoglucanase liberate cellobiose from the ends of cellulose chains (reducing and non-reducing) and β-glucosidases will further liberate glucose from cellobiose [30,31]. Xylanase catalysis xylan hydrolysis to produce a mixture of xylose, xylobiose and shorter xylo-oligosaccharides [32]. Bacterial isolates with ability to produce cellulase and xylanase enzymes can be used as good candidates in lignocellulosic materials bioconversion, and contributing in meeting the demand for cellulase and xylanase enzymes for various applications in different markets.

Some bacterial genera such as *Bacillus, Thermonospora, Bacteriodes, Acetivibrio, Clostridium, Paenibacillus, Erwinia* and *Ruminococcus* were found to have cellulolytic and/or xylanolytic activities [14,33-35]. Members of the genus *Paenibacillus* such as *P. curdlanolyticus*, *P. macquariensis*, *P. cellulosilyticus*, and *P. barcinonensis* were previously reported to have cellulase and/or xylanase activities [35-39]. However, *P. illinoisensis* has not hitherto been reported as producing cellulolytic and xylanolytic enzymes. The ability of *P. illinoisensis* CX11 to produce cellulolytic and xylanolytic enzymes was tested using SSF of corn stalk to determine the production of CMCase, xylanase, FPase, avicelase as well as extracellular protein. Results showed that *P. illinoisensis* CX11 can produce CMCase (400.12 ± 1.23 U/L), xylanase (385.57 ± 2.25 U/L), FPase (266.93 ± 2.22 U/L), avicelase (187.85 ± 2.22 U/L) and extracellular protein (4.56 ± 0.14 mg/l) (Fig. 1.). This indicates that *P. illinoisensis* CX11 has the ability to produce enzymes that are required for the saccharification of lignocellulosic materials. Although the production of CMCase, xylanase, FPase and avicelase was previously reported in few members of *Paenibacillus* such as *P. polymyxa*, *P. terrae* ME27-1, and *P. curdlanolyticus*

B-6 [40,41,36], the current study is the first to report *P. illinoisensis* CX11 as CMCase, xylanase, FPase and avicelase producing species.

In this study, corn stalk, wheat bran, sawdust, and corn cob were used to test the ability of *P. illinoisensis* CX11 to saccharify lignocellulosic materials. Results showed that *P. illinoisensis* CX11 exhibited various abilities to saccharify corn stalk, wheat bran, sawdust, and corn cob with total reducing sugar of 2.5 ± 0.02, 2.59 ± 0.03, 2.07 ± 0.16, and 3.31 ± 0.09 mg/l for TRS-G and 2.58 ± 0.09, 2.51 ± 0.37, 1.86 ± 0.16, and 3.29 ± 0.2 mg/l for TRS-X, respectively (Fig. 2.). This was attributable to the ability of *P. illinoisensis* CX11 to produce the required cellulase and xylanase enzymes for the saccharification process (Fig. 1.). *Paenibacillus* genus contains some species such as *P. campinasensis* BL11 and *P. curdlanolyticus* B-6 that were reported to have the ability to degrade lignocellulosic materials [42,43], or *P. polymyxa* BEb-40 which was reported as a promising bacterial strain for the production of endoglucanases, with possibilities of application in the breakdown of lignocellulosic biomass [44]. However, *P. illinoisensis* has not been reported as having the ability to hydrolyze different lignocellulosic materials.

The demand for microbial industrial enzymes has been increasing due to their potential for use in different applications for a variety of industrial processes [45]. Despite continuing efforts to find an effective means for using enzymes for the bioconversion of lignocellulosic materials into high value chemicals as well efforts to find cost-effective methods to produce the required enzymes, there is still a bottleneck in the process. Therefore, the continuing search for new bacterial isolates with the ability to produce cellulolytic and xylanolytic enzymes could provide further improvement in the use of microbial enzymes in bioconversion processes. Thus, the results presented in this study indicate that *P. illinoisensis* CX11 could be a good source of enzymes that can be used in the saccharification of cheap and renewable lignocellulosic materials to produce high value chemicals and products.

4. CONCLUSION

Paenibacillus illinoisensis CX11 offers good prospects for the production of CMCase, xylanase, FPase, and avicelase enzymes which can be applied in the process of converting the lignocellulosic materials into their respective fermentable sugars. This step is critical prior to converting the resulting fermentable sugars into the targeted compounds. To the best of the authors' knowledge, this study is the first to report *P. illinoisensis* CX11 as having the ability to hydrolyze different lignocellulosic materials (corn stalk, wheat bran, sawdust, and corn cob) and can be considered a good candidate for CMCase, xylanase, FPase, and avicelase enzymes that can be used in the saccharification step of lignocellulosic materials.

5. ACKNOWLEDGMENTS

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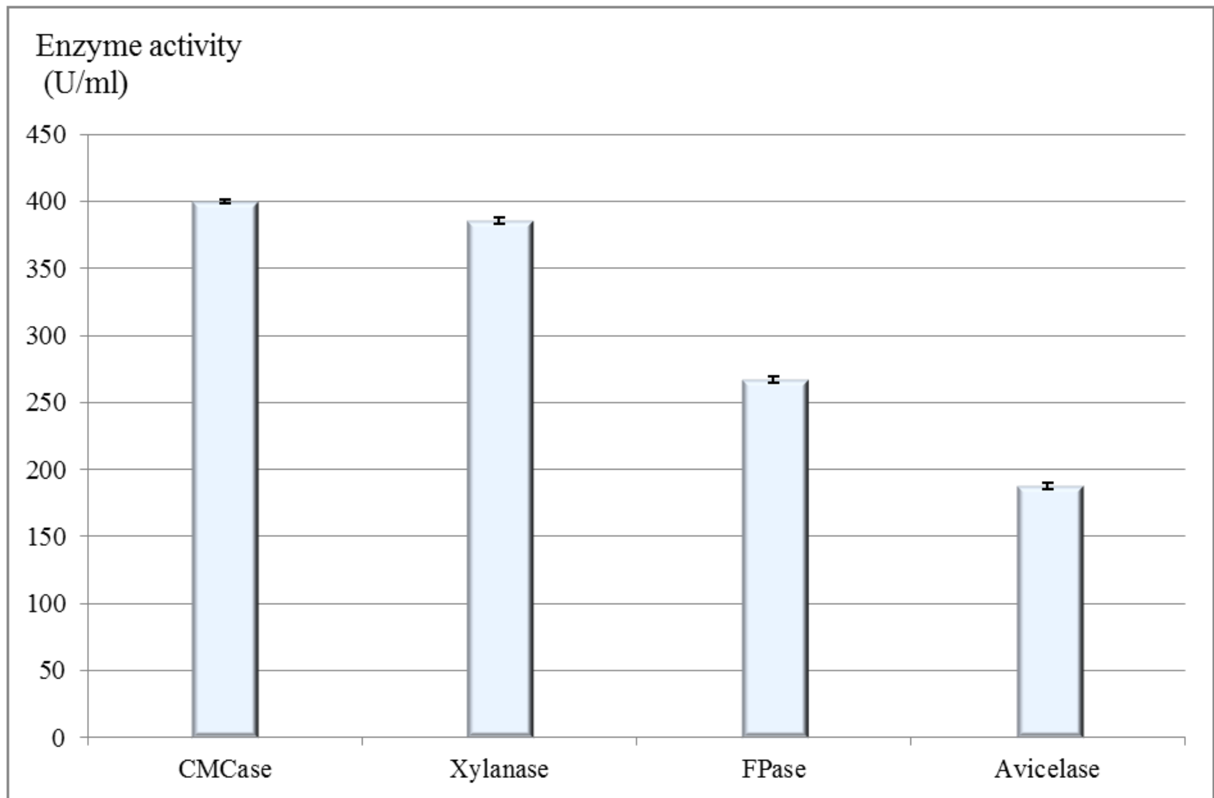

Figure 1: The determination of CMCase, xylanase, FPase, avicelase and extracellular protein of *P. illinoisensis* CX11 using SSF of corn stalk.

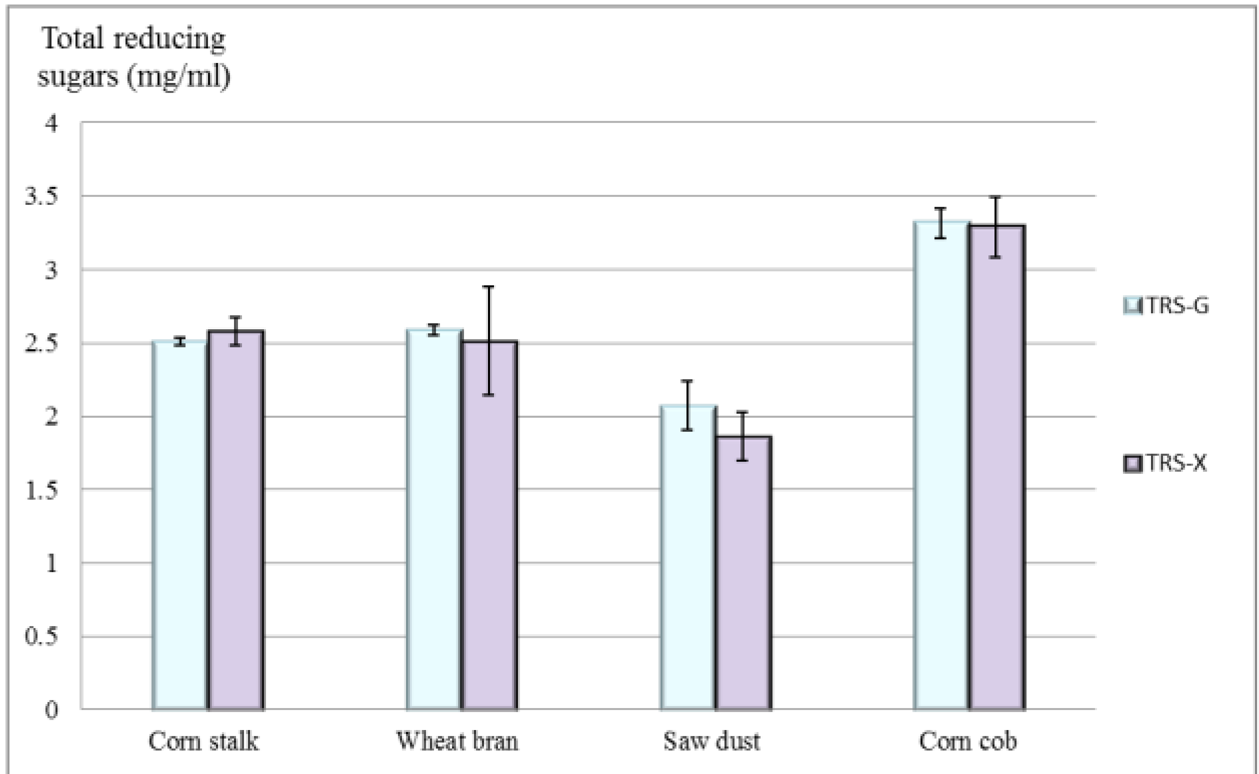

Figure 2: Total reducing sugars from corn stalk, wheat bran, sawdust, and corn cob saccharified by *P. illinoisensis* CX11.