Increasing xylanase activity of *Bacillus subtilis* by atmospheric pressure plasma jet for biomass hydrolysis

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Abstract

Xylanase producing bacteria, *Bacillus subtilis*, was bombarded by an atmospheric pressure plasma jet (APPJ) and screened for higher catalytic activity. The bacteria were bombarded with plasma of argon or helium with energy of 120 W for a duration of 1–5 min. A mutant with higher xylanase activity was observed under argon plasma treatment at 1 min on media containing xylan as substrate. Subsequently, the xylanase gene from the mutant was sequenced and named *MxynA*. Sequence analysis revealed only a single missense mutation on the *MxynA* gene causing amino acid substitution from threonine to serine at position 162 (T162S) within the xylanase protein of the mutant. Consequently, *MxynA* was subcloned into expression vector, pETDuet-1 under T7 promoter and expressed in *E. coli* BL21 (DE3). The optimum temperature and pH of *MxynA* and its parent expressed in *E. coli*, named *CxynA* were 60 °C and pH 5, respectively. Moreover, *MxynA* showed higher xylanase activity approximately 4 fold higher than that of the control upon a wide range of pH and temperature conditions. From kinetic parameters analysis, the mutant showed higher enzyme turnover (k_{cat}) than the control. The hydrolysis ability of the *MxynA* enzyme on lignocellulosic wastes, such as rice straw, corncob and para grass was investigated using the released reducing sugar as an indicator. The *MxynA* enzyme showed a greater amount of reducing sugar released from all lignocellulosic wastes other than the control, particularly from para grass. This study demonstrated that the T162S mutation possibly improved the catalytic efficiency of *MxynA*.

Keywords Mutation · Atmospheric pressure plasma jet (APPJ) · *Bacillus subtilis* · Xylanase · Agricultural waste

Introduction

Annually, tons of agricultural wastes such as rice straw, corn husk, corncob, and para grass are found in many agricultural areas in Thailand. To eliminate these wastes, burning is an option, but it causes air pollution. Therefore, converting

lignocellulosic wastes for animal feed has become desirable common over the last decade. Animal feed from lignocellulosic wastes not only lessens environmental pollution, but also reduces feed cost. Since lignocellulosic components in plant cell walls consist of cellulose (45–55%), hemicelluloses (25–35%), and lignin, these components can be used as raw materials for compost fertilizer and fermented feed for animals. Generally, agricultural wastes such rice straw and corn husk are used as raw materials for cattle feed. The fermentation process feeds naturally occurring bacteria to partially break the raw materials using their enzymes. Consequently, cellulase and xylanase producing bacteria are selected for the fermentation process using these raw materials for cattle feed. For example, xylanase was applied in a forage pre-treatment process to improve digestibility of ruminant feed (Gilbert and Hazlewood 1993). These enzymes break down xylan polymers in the ingredients of feed (Twomey et al. 2003). In addition, feeding poultry with mixed cellulolytic enzymes (xylanase, protease, and amylase) exhibited significantly higher body weights and greater

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net energy (Café et al. 2002). Further, dairy cattle fed with cellulase and xylanase showed an increase in dry matter intake and milk production (Romero et al. 2016). This evidence indicates that xylanase has an important role in animal feed production. During the feed fermentation process, more stable and higher catalytic activity enzymes under a broad range of pH and temperature are required (Schroeder 2004). Considering that most available xylanases do not meet the requirement for feed fermentation and industrial applications, xylanases with both abilities are sought.

Atmospheric pressure plasma jet (APPJ) is an alternative technique to apply for enzymatic improvement. A plasma is an ionized gas with an equal number of positive and negative charged particles. Plasma can be generated by various methods such as photo ionization, heat radiation, gas discharge, and radio frequencies (Liao et al. 2017). According to the relative energetic levels of electrons, plasma can be categorized into two main groups, high-temperature plasma and low-temperature plasma (also called gas discharges) (Fridman et al. 2005). In recent years, APPJs have been frequently applied in biological research such as sterilization of both biotic and abiotic surfaces (Laroussi 2015), the impact of plasma on DNA damage (Lee et al. 2014; O'connell et al. 2011), to improve amylase activity in *Bacillus subtilis* (Ma et al. 2016), and the increase of carboxymethyl cellulase enzyme in *Trichoderma viridae* (Xu et al. 2011). These studies indicate that APPJ is a novel technique that has the potential to be used in a wide range of applications in the biotechnology field, including developing enzyme properties for environmental concerns. The aim of this work is to enhance the xylanase activity of *B. subtilis* by inducing mutation using plasma jet and to apply this enzyme in animal feed production. This is the first reported instance of a bacterial mutant resulting from plasma jet technique to be used as animal feed using agricultural wastes as raw materials.

Materials and methods

Bacterial strains, growth conditions, and vectors

Xylanase producing bacteria, *B. subtilis*, was isolated from termite comb. *Escherichia coli* strains DH5 α and BL21 (DE3) were used as hosts for transferring plasmids. All bacterial strains were grown at 37 °C in Luria–Bertani (LB) broth with vigorous shaking at 120 rpm. Cloning and expression vectors, pTZ57R/T and pETDuet-1, were purchased from Thermo Fisher Scientific (USA) and Novagen (USA).

Atmospheric pressure plasma jet bombardment

Plasma bombardment of the bacterial samples was carried out using our in-house developed atmospheric pressure

plasma jet (APPJ) at Biotechnology Unit, University of Phayao. A schematic of the APPJ system, comprised of a high-voltage power supply, electrodes, and a dielectric tube, is shown in Fig. 1.

B. subtilis cells were harvested by centrifugation at 6000 rpm for 5 min after overnight incubation at 37 °C then resuspended in 100 μ l of sterile distilled water. Bacterial suspensions were dropped in holes of a sample holder for plasma bombardment. Both argon and helium plasmas were chosen to generate plasma with an energy input of 120 W and gas flow rate of 2 SCCM (standard cubic centimeters per minute) at room temperature for all experiments. The bacterial samples were bombarded at time intervals ranging from 1 to 5 min. After plasma bombardment, 1 ml of LB media was added to each bombarded sample and incubated at 37 °C on a rotating shaker for 1 h. Then, 100 μ l of each sample was spread on LB agar containing 1% (w/v) beechwood xylan and incubated overnight at 37 °C.

Bacterial survival comparison

After bombardment, all bacterial samples were recovered by spreading them on LB agar plates and incubated at 37 °C. The number of colonies was counted to determine the colony forming units (CFUs). The effectiveness of the bombardment was calculated as the percentage of CFUs observed on treated plates, relative to CFUs on untreated plates at the lowest dilution, where survival was observed (Raheem and Mahmood 2013). The percentage of bacterial survival was calculated using the following:

Fig. 1 Schematic drawing of the atmospheric pressure plasma jet (APPJ) system

$$R = \frac{N_0 - N_t}{N_0} \times 100\%,$$

where, N_0 is the C.F.U. of non-treated bacterial control and N_t is the C.F.U. of treated bacteria.

Screening xylanase (*xynA*) gene mutation

After the bacterial cells were bombarded with either argon or helium plasma, the bombarded bacterial samples were screened for xylanase (*xynA*) gene mutation by spotting on agar plates consisting of 1% (w/v) beechwood xylan and incubated at 37 °C for 24 h. Wild type *B. subtilis* was grown on the same agar media as the control. The hydrolysis halos were stained by Congo red to observe bacterial xylanase activity (Hoyt and Sizemore 1982). The qualitative xylanase activity of each bombarded bacterial was evaluated by the ratio between hydrolysis halo diameter to the colony diameter ratio (H/C). The mutant with the highest H/C ratio was selected with the xylanase gene named *MxynA* and the control *CxynA*.

Gene cloning and vectors construction

Xylanase gene cloning

Chromosomal DNA of control bacteria and the mutant were extracted and used as a DNA template for xylanase gene cloning. A known sequence of *B. subtilis* xylanase gene in GenBank (accession no. WP_038829967.1) was chosen as a template to design primers for amplifying the xylanase gene. Two primers, forward primer 5'ccggcaggatccgcaggacaaaaacgccag3' with restriction site *Bam*HI and reverse primer 5'ctaataagcttgcttaagcttaactaatt3' with restriction site *Hind*III were designed. Then entire xylanase gene sequences of *CxynA* and *MxynA* were amplified by PCR. The 642 bp PCR products were obtained and subcloned into the corresponding sites of pTZ57R/T vector. DNA sequencing was performed using an autosequencer (ABI) by Macrogen, Korea and the nucleotide sequences were determined online with NCBI blast (<https://www.ncbi.nlm.nih.gov/>).

Construction of recombinant expression vectors and transformation of *E. coli* BL21 (DE3)

Both *CxynA* and *MxynA* were digested with *Bam*HI and *Hind*III and ligated into the corresponding sites of pETDuet-1 vector. The recombinant plasmids of the mutant and the control, named p*MxynA* and p*CxynA*, were transformed into *E. coli* BL21 (DE3), respectively. The transformed cells were cultured on LB plates containing 100 µg/ml of ampicillin and incubated overnight at 37 °C.

Expression and purification of recombinant *MxynA* and *CxynA* in *E. coli* BL21 (DE3)

The control and the mutant transformants were cultured in LB-ampicillin (100 µg/ml) medium at 28 °C with vigorous shaking at 200 rpm until the OD_{600} reached 0.6. The expression of the recombinant xylanase was induced with Isopropyl β-D-1-thiogalactopyranoside (IPTG, 1 mM) for continuous 12 h cultivation at 28 °C. The bacterial cells were harvested by centrifugation followed by ultrasonication. Then both *MxynA* and *CxynA* were purified using Protino, Ni-TED 1000 kit (Macherey-Nagel, Germany), according to the manufacturer's instructions. Protein concentration of the purified xylanase was measured using Bradford method with bovine serum albumin as standard.

Structural analysis between *MxynA* and *CxynA*

Three-dimensional (3-D) structures of the *MxynA* and *CxynA* were developed by homology modeling, a computational tool that has been widely applied in the field of structural biology. The three-dimensional structure was generated by SWISS-MODEL protein modeling server (<https://swissmodel.expasy.org/>).

Characterization of *MxynA* and *CxynA*

Xylanase activity of *MxynA* and *CxynA* was assayed with 1% (w/v) of beechwood xylan at various temperatures ranging from 10 to 100 °C for 15 min. The amount of reducing sugar was determined by DNS method (Miller 1959). One unit of enzyme activity was defined as the amount of enzyme that liberated one nanomol of reducing sugar per minute. The optimum pH values of *MxynA* and *CxynA* were determined ranging from pH 2.0 to 11.0 at 50 °C for 15 min.

Enzyme kinetic assays

Kinetic parameters were determined using beechwood xylan as substrates at concentrations in the range of 0.1–10 mg/mL. The reaction mix contained 25 µg/mL of enzyme, 0.5 mM citrate buffer pH 6.0, and incubated at 50 °C for 15 min. The amount of reducing sugar released was measured using DNS method. The K_m , k_{cat} , and k_{cat}/K_m were obtained from Lineweaver–Burk plots (Lineweaver and Burk 1934).

Hydrolysis of xylanase on lignocellulosic raw materials

Xylanase hydrolysis activities of *MxynA* and *CxynA* were investigated using lignocellulosic material, including rice straw, corncob, and para grass. The hydrolysis activities were carried out by incubating the purified enzyme in 5%

(w/v) of each material at 37 °C for 7 days. The suspension from the reaction was collected every 24 h to measure the reducing sugar released from each material by the DNS method.

Results

Atmospheric pressure plasma jet induced mutation in bacterial cells

B. subtilis was bombarded under various conditions by the plasma jet, then the survival of each treated bacteria was investigated using a plate count method and compared with the bacterial control. Higher survival inactivation efficiencies were observed in helium plasma treatment at 1 and 2 min treatments. The highest survival inactivation was observed at 5 min treatment with argon plasma (Fig. 2). To screen for bacterial mutants, the bombarded bacteria were spread on media containing 1% (w/v) beechwood xylan. The mutant with the greatest xylanase activity showed the highest *H/C* ratio on a plate containing beechwood xylan, observed only under argon plasma bombardment at 1 min (Fig. 3).

Cloning of the xylanase gene and sequence analysis

The entire *CxynA* and *MxynA* were amplified by PCR using the specific primers (as describe in materials and methods), subsequently cloned into pTZ57R/vector and sequenced. Both *CxynA* and *MxynA* nucleotide sequences consisted of 642 nucleotides encoding for 213 amino acids. Nucleotide alignment between *CxynA* and *MxynA* revealed that five nucleotide substitutions were found in *MxynA* (data not shown), but only one amino acid residue changed in the *MxynA* sequence.

The amino acid comparison between *CxynA* and *MxynA* showed that these two sequences were almost identical

Fig. 2 Survival rate comparison of bacteria bombarded by atmospheric pressure plasma jet using helium and argon plasma

Fig. 3 Congo red staining displayed hydrolysis halo zone of *B. subtilis* **a** control and **b** mutant

(Fig. 4), except that the threonine residue was substituted by serine residue at position 162 (T162S). Based on the amino acid sequence homology, our xylanases belong to the glycosyl hydrolase family 11 (GH11). Generally, the structure of GH11 xylanases consist of two large β -pleated sheets and single α -helix that form a structure resembling a partially closed right hand (Törrönen and Rouvinen 1997). To investigate the structural changes responsible for the improved catalytic activity of *MxynA*, the 3D structures of *MxynA* and *CxynA* were simulated by SWISS-MODEL protein modeling webserver (<https://swissmodel.expasy.org/>). The template search for homology modeling was performed by BLAST program. From our results, a GH11 xylanase from *B. subtilis* with PDB no. 2DCY was chosen as the template for structural analysis. Based on sequence alignment and domain analysis, T162S residue was located at a loop connecting the β -sheet B7 and A6 exposed to a hydrophilic environment (Fig. 5). The former β -strand constitutes an active site and carries the conserved residues. For instance, Phe¹⁵³ and Trp¹⁵⁷ maintain the substrate during the reaction mechanism (Murakami et al. 2005).

Characterization comparison between the xylanase enzymes

Effect of pH and temperature on xylanase activity

The effects of pH and temperature on xylanase activity were determined. The purified *MxynA* and *CxynA* were incubated with 1% beechwood xylan and a pH ranging from 2.0 to 11.0 at 50 °C for 15 min. The quantity of released reducing sugar was detected and calculated using DNS method. The catalytic activity of *CxynA* and *MxynA* at pH 5.0 was 304.10 IU/mg and 1463.47 IU/mg, respectively (Fig. 6).

Fig. 4 Sequence comparison of xylanase of the wild type (CxynA) and the mutant (MxynA) using CLUSTALW program. The mutant residue (T162S) is indicated by the red arrow

CxynA	MFKFKKNFLVGLSAAALMSISLFSATASAASTDYWQNWDGGGIVNAVNGSGGNYSVNWSN	60
MxynA	MFKFKKNFLVGLSAAALMSISLFSATASAASTDYWQNWDGGGIVNAVNGSGGNYSVNWSN	60

CxynA	TGNFVVGKWTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTG	120
MxynA	TGNFVVGKWTGSPFRTINYNAGVWAPNGNGYLTLYGWTRSPLIEYYVVDSWGTYRPTG	120

CxynA	TYKGTVKSDDGGTYDIYTTRYNAPSIDGDRITFTQYWSVRQTKRPTGSNATITFSNHVNA	180
MxynA	TYKGTVKSDDGGTYDIYTTRYNAPSIDGDRITFTQYWSVRQSKRPTGSNATITFSNHVNA	180

CxynA	WKSHGMNLGSNWAYQVMATEGYQSSGSSNVTW	213
MxynA	WKSHGMNLGSNWAYQVMATEGYQSSGSSNVTW	213

Fig. 5 The ribbon representation of the three-dimensional structures of CxynA (a) and MxynA (b) shows the position of the mutation (T162S). Hydrophobic residues located at β -sheet B7 involved in substrate binding are shown as yellow sticks. The model was generated with SWISS-MODEL server using family 11 xylanase from *Bacillus subtilis* (PDB code: 2DCY) as the template

Fig. 6 Xylanase activities of MxynA and CxynA at different pH ranging from pH 2.0 to 11.0 at 50 °C

Furthermore, both MxynA and CxynA showed their similarity of activities in a wide pH range of 4.0–8.0.

For optimal temperature, the catalytic activity of both xylanases was carried at a broad range of temperatures.

Both MxynA and CxynA had an optimum temperature of 60 °C. The catalytic activity of MxynA was approximately 2 fold that of CxynA over a broad range of temperatures from 10 to 100 °C. Moreover, the catalytic activity of MxynA still retained about 80% at 90 °C after 15 min of incubation (Fig. 7).

The kinetic parameters of the purified enzymes CxynA and MxynA toward xylan were determined to reveal the effects of the substitution of Thr to Ser at position 162 on enzyme activity. As shown in Table 1, the K_m value of MxynA was not significantly different from that of CxynA. Whereas, the k_{cat} and k_{cat}/K_m values of MxynA were approximately twofold that of CxynA. These results suggest that mutation leads to a higher turnover number.

Fig. 7 Xylanase activities of MxynA and CxynA at different temperatures ranging from 10 to 100 °C

Table 1 Kinetic parameters on beechwood xylan of CxynA and MxynA

Kinetic parameters ^a	K_m (mg/mL)	k_{cat} (/s)	k_{cat}/K_m (/s/mg/mL)
CxynA	9.11	15.43	1.69
MxynA	10.8	35.19	3.26

^aValues are the mean of three replicates

Fig. 8 The hydrolysis activity of each substrate from day 1 to day 7

Lignocellulosic biomass hydrolysis by xylanase

Since MxynA exhibited enzyme activity about 4 times higher than CxynA over a broad range of temperatures and pH, both MxynA and CxynA were applied to hydrolyze lignocellulosic wastes, including rice straw, para grass, and corn cob. The reducing sugars released from each hydrolysis reactions were then measured. Reducing sugars were detected from the first day of incubation, increasing gradually until the end of day 7 (Fig. 8).

Discussion

Survival comparison of bacteria bombarded by helium and argon plasma

The effect of argon and helium plasma bombardment on *B. subtilis* survival is shown in Fig. 2. The survival at 1 min of bombardment reveals that helium plasma has a significantly different and greater effect than argon plasma. When exposure times were increased, the effect of argon plasma on survival dramatically decreased, while the effect of helium plasma gradually decreased. At 5 min exposure, survival under argon bombardment was barely detected. These results indicate that the longer the exposure to plasma bombardment, the lower the bacterial survival. Previous research has reported argon plasma having a greater effect on inactivate bacterial cells than helium plasma (Chandana et al. 2018; Lee et al. 2014). The different effects on bacterial survival between helium and argon plasma could be due to the physical and chemical characteristics of argon and helium gases, especially the different ionization energies and molecular weight of ions. The penetration depth of light ions such as helium, was higher than that of heavy ions like argon, at low energy levels or shorter exposure times (Cai et al. 2006). When exposure time was increased, the penetration of argon ions into cells was deeper. In addition, heavy ions have higher linear energy transfer (LET) than light ions, which causes a greater relative biological effect (RBE) (Bai et al. 2016). Heavy ions with a different LET spectrum killed more cells by inducing DNA double strand breaks (DSBs), which are more difficult to repair (Aufderheide et al. 1987; Lobrich et al. 1994). Therefore, 5 min exposure to argon bombardment led to more cell damage than the light ion, helium.

Characterization comparison between the xylanase enzymes

After plasma bombardment, the mutant, MxynA, with the highest xylanase activity was screened under argon plasma bombardment. According to the above experimental data, the recombinant MxynA showed a higher xylanase activity, approximately 2 fold, under a wide range of temperature conditions. Catalytic activity of MxynA was about 3.5–3.9 fold higher than that of the control at pH 4–6. During the fermentation process, pH values decreased rapidly and usually kept under pH 4–5 (Stefanie et al. 2000). Meanwhile, the temperature increased gradually up to 42 °C (McDonald et al. 1966). Therefore, enzymes which had a high catalytic activity between 40 and 50 °C were

required. Since the xylanase activity of MxynA displayed high hydrolysis characteristics over a wide pH range 4–8 and a broad temperature range 20–60 °C (Figs. 6, 7), this enzyme is suitable for livestock feed fermentation.

The kinetic parameters analysis of the purified enzymes CxynA and MxynA toward beechwood xylan were performed. It was discovered that the mutant had a similar K_m to the control, but a significantly increased k_{cat} (Table 1). Apparent k_{cat} and k_{cat}/K_m values of MxynA were 35.19/s and 3.26/s/mg/mL, respectively, while k_{cat} and k_{cat}/K_m values of CxynA were 15.43/s and 1.69/s/mg/mL, respectively. The higher values of turnover number (k_{cat}) indicated an improved catalytic efficiency. These results suggest that mutation plays a significant role in enzyme action. To elevate, the role of serine at position 162 (Ser¹⁶²) from the viewpoint of conformation, the homology structure model of MxynA was generated using GH11 xylanase from *B. subtilis* (PDB No. 2DCY) as the template. The structural model reveals that Ser¹⁶² is located on the loop connected to the β -sheet B7 and A6 exposed to a hydrophilic environment (Fig. 5). To the best of our knowledge, there is no report on the role of an amino acid at this loop in substrate binding and enzyme catalysis. However, we found that the replacement of Thr with Ser increased the catalytic efficiency of enzyme (Table 1). According to amino acid sequence alignment shown in Fig. 9, the amino acid equivalent of Ser¹⁶² can be substituted to Thr, Asn, and Gln, which are polar and usually contribute to the formation of hydrogen bonds with water molecules (Murakami et al. 2005). As shown in Fig. 5, the hydroxyl group of Thr¹⁶² in CxynA and Ser¹⁶² in MxynA was similarly oriented to protein surfaces to form hydrogen

bonds with water molecules. However, Thr¹⁶² of CxynA possesses a methyl group with more hydrophobicity, which was also exposed to the protein surface, so it might be less favorable to create an interaction with water compared to Ser of MxynA. Therefore, this enhancement of the enzyme catalysis may be explained by the fact that Ser substitution conduce more hydrophilic interactions of enzyme and substrate with increased turnover of the enzyme. Consequently, it might contribute to the enhancement of the enzyme catalysis. Moreover, the substituted position occurred at highly conserved residues QYWSVRXXXR (X corresponds to a non-conserved residue). This phenomenon might indicate that these conserved sequences may play an important role in catalytic activity by supporting or involving the enzyme binding to polymeric xylan. For further characterization, multiple sequence alignment was carried out and the result clearly informs that our mutation residue belongs to the ‘knuckles’ of the enzyme, which is reported to be a secondary substrate binding site (SBS) of *B. circulans* (Cuyvers et al. 2011).

Lignocellulosic biomass hydrolysis by xylanase

For lignocellulosic waste hydrolysis, three kinds of biomass were used as substrates. The highest reducing sugar was liberated from para grass after 7 days (62.54 mg/g substrate) of incubation with xylanase produced from MxynA. According to the composition of lignocellulosic biomass, which contains 25–35% of hemicellulose, the reducing sugar released during our experiment can be calculated as 10–25% of total hemicellulose in each lignocellulosic substrate. This result

Fig. 9 Amino acid sequence alignment of small molecular mass xylanases (generally less than 30 kDa). The sequence alignment is based on structure considerations. The sequences are *B. cir.*=*B. circulans* (accession no. AYV73613.1); *T. com.*=*Thermobacillus composti* (accession no. WP_015253740.1); *C. cel.*=*Clostridium cellulolyticum* (accession no. WP_015925319.1); *B. amy.*=*Bacillus amyloliquefaciens* (accession no. ADK92885.1); *B. kawa.*=*Asper-*

gillus kawachii (accession no. BAA07264.1); *T. harz.*=*Trichoderma harzianum* (accession no. AIK67330.1); *T. ree.*=*Trichoderma reesei* (accession no. APU51339.1). The conserved residue QYWSVRXXXR are indicated by yellow highlighting. Residues variable within polar amino acid group are displayed as blue letters on a red box

indicates that the mutant xylanase can be used to hydrolyze these lignocellulosic wastes without a pre-treatment process. Therefore, lignocellulosic wastes could be used for cattle feed, particularly corncob and para grass by fermentation using MxynA. Additionally, cattle feed from fermented corncob with the mutant xylanase has been primarily given to dairy cows in Phayao province (unpublished data). These results suggest that the APPJ is a reliable tool for inducing mutation in bacteria to increase enzyme activity for animal feed production. This can reduce animal feed costs and air pollution from the burning lignocellulosic waste.

Conclusions

B. subtilis was bombarded by argon plasma to enhance its xylanase activity. It was found that the bacterial mutant, which exhibited the highest xylanase activity was obtained with a 1 min bombardment duration. The mutant named MxynA showed a single amino acid residue substitution from threonine to serine (T162S) at beta sheet linker and very close to the C terminal. The recombinant MxynA, showed higher catalytic efficiency, approximately 4 fold the control under a broad range of pH and temperatures. In addition, xylanases' hydrolysis ability on agricultural wastes such as rice straw, corncob and para grass was also demonstrated and para grass released the highest amount of reducing sugar. To the best of our knowledge, improving the hydrolysis characteristics of xylanase by plasma mutation at the beta sheet linker region leads to an increase of enzyme turnover (k_{cat}) that has rarely been reported.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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