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Original articles

HYDROLYSIS OF PLANT BIOMASS USING RECOMBINANT A-AMYLASE FROM *BACILLUS LICHENIFORMIS* AND XYLANASE FROM *BACILLUS SONORENSIS*

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ABSTRACT

With the reduction of fossil hydrocarbon sources and the increasing need for energy, chemical resources and food, the use of biomass as a renewable source is of great importance. This, in turn, necessitates efficient enzymes for bioconversion of biomass. Enzymes are important in industry because they are biocatalysts for complex chemical processes. Conversion of plant biomass into sugars requires a complex of enzymes of a certain composition, which corresponds to the type of biomass and the method of pretreatment. The efficiency of enzymatic hydrolysis can be increased by optimizing the composition of the enzymatic complex, increasing the catalytic activity and stability of the enzymes included in it. A large number of microbial amylases are now commercially available, and they have almost completely replaced chemical starch hydrolysis in the processing industry. The use of bacterial xylanases is a key step in the conversion of lignocellulosic polysaccharides into fermentable sugars for the production of biofuels and value-added products. Enzyme complexes containing both α-amylases and xylanases have found applications in the pulp and paper industry and feed production.

Recombinant α-amylase and xylanase from *Bacillus licheniformis* and *Bacillus sonorensis*, respectively, were obtained using recombinant DNA technology. These enzymes were purified by metal affinity chromatography from the lysate of induced cultures of recombinant strains. Enzymatic hydrolysis of potato starch and birch xylan with these recombinant enzymes was performed. The hydrolysis products of these polysaccharides were studied by thin-layer chromatography. It was found that the products of hydrolysis of potato starch using recombinant α-amylase were maltose and maltooligosaccharides and a small amount of glucose. Xylan was isolated from birch sawdust and hydrolyzed by recombinant xylanase. Detection of the hydrolysis products of xylan showed that xylan is hydrolyzed to oligoxylans with a degree of polymerization of at least 2 monomers. No xylose was detected among the hydrolysis products. The results indicate that α-amylase from *Bacillus licheniformis* and xylanase from *Bacillus sonorensis* are endoenzymes.

Keywords: alpha-amylase, xylanase, starch, xylan, *Bacillus sp*.

INTRODUCTION

Carbohydrate polymers are the main components in plant biomass and represent either nutrients (starch) or mechanical strength (cellulose and hemicellulose). In nature, a heterogeneous aggregate of different polysaccharides forms dense microfibrils that are not amenable to enzymatic cleavage. For example, seeds in which starch is surrounded by layers of other polysaccharides such as cellulose and pectin, which has a dense structure and is protected from the influence of animal gastrointestinal enzymes [1]. The peculiarities in the chemical structure of polysaccharides limit the ability of hydrolyzing enzymes, which manifests itself in the selective action of most carbohydrases on the carbohydrate substrate.

Therefore, several monofunctional enzymes must be used to hydrolyze multicomponent carbohydrates such as lignin. In particular, glycoside hydrolases such as endoglucanases, exoglucanases, β-glucosidases, and xylanases are used to degrade lignocellulosic biomass [2].

Due to the reduction of fossil sources of hydrocarbons and energy, and the increasing need for energy, chemical resources and food, the use of biomass as a renewable source has become very important. This, in turn, causes the need for efficient enzymes for bioconversion of biomass [3]. Amylases are enzymes that hydrolyze starch. The high efficiency of amylolytic enzymes allows them to compete in industry with chemical starch hydrolysis technology. The high demand for α-amylase has resulted in α-amylases accounting for 30% of the total enzyme market. Alpha-amylases cleave the α-(1,4)-D-glucoside bond in starch and other related polysaccharides to form simple sugars: glucose and maltose [4]. Xylan, as the main component of hemicellulose, forms the cell wall of plants and is found in both deciduous and coniferous plants. Xylanases are a group of hydrolytic enzymes that cleave the β-1,4 glycoside backbone of xylan to form xylooligosaccharides: xylose and xylobiose [5]. Microbial xylanases, which have a number of biotechnological properties and are used for juice and beer clarification, bakery production, kraft pulp bleaching, vegetable fiber refining, tobacco processing, extraction of vegetable oils, textile fiber recovery, bioconversion of agricultural waste and biofuel production, are in demand for use in industrial processes [6,7]. α-amylases and xylanases of microbial origin have several advantages associated with their high specific activity and cheap industrial production [8-10]. During the processing of plant material, a large amount of waste products is formed, including lignocellulosic waste products. Recent studies have focused on studying the mechanism of action of hydrolytic enzymes to form oligosaccharides. Complete hydrolysis of plant (lignocellulosic) biomass requires the selection and study of enzymes acting at the same reaction conditions.

The aim of this work was to hydrolyze starch and xylan polysaccharides using α-amylase from *Bacillus licheniformis* and xylanase from *Bacillus sonorensis*. The strains were isolated from soil near Taraz city, and previously, α-amylase and xylanase were studied [11,12]. These enzymes showed good hydrolyzing properties against plant polysaccharides.

This work is a continuation of the studies initiated and is devoted to the study of the degree of starch and xylan hydrolysis using these enzymes. To achieve the aim α-amylase and xylanase were obtained in recombinant form in *Escherichia coli* cells. The recombinant enzymes were purified and studied. The products of enzymatic hydrolysis of potato starch and birch xylan were examined by thin-layer chromatography (TLC) using appropriate standard samples of mono- and oligosaccharides.

MATERIALS AND METHODS

Media, chemical reagents

E.coli strains were cultured in Lennox broth medium (Broth-Lennox, LB, 0.5% yeast extract, 1% peptone, 0.5% NaCl). Potato starch produced by Sigma-Aldrich was used as a substrate for alpha-amylase and beech xylanase produced by Megazyme. Mono- and oligosaccharide standards for thin-layer chromatography: glucose, maltose, maltotriose, maltotetraose, maltopentaose, xylose, xylobiose, xylothraose, xylopentaose, and xylohexaose from Megazyme.

Expression of α-amylase and xylanase genes in *Escherichia coli* cells

The α-amylase gene (1536 bp) was amplified from the genomic DNA of *B. licheniformis* strain and cloned into the pET-28c(+) vector at the NdeI and NotI sites. The xylanase gene (555 bp) was amplified from the genomic DNA of *B. sonorensis* strain and cloned into vector pET-28c(+) at the NcoI and BamHI sites. In the resulting pET-28c(+)/Amy and pET-28c(+)/Xyn constructs, the α-amylase and xylanase genes were inserted under the control of the T7 promoter, which is activated in the presence of isopropyl-β-d-1-thiogalactopyranoside (IPTG).

Escherichia coli strain ArcticEspress(DE3)RP cells were transformed by electroporation with these plasmid vectors. The resulting kanamycin-resistant transformants were cultured in 1 liter of LB broth with kanamycin (50 μg/ml) at 18°C and 150 rpm. In the middle of the logarithmic growth phase (OD_{600} = 0.6), IPTG was added to a final concentration of 0.5 mM and incubated for 16 hours.

Purification of recombinant alpha-amylase and xylanase

After induction with IPTG, cells were harvested by centrifugation at 6000 g for 7 min at 4°C. The cells were suspended in 20 mM Tris-HCl (pH 8.0) with 500 mM NaCl and lysed with lysozyme (2 mg/ml) followed by ultrasound treatment. The lysate was clarified by centrifugation (40000×g, 1 h, 4°C) and filtered through a 0.45-μm filter. The recombinant protein (α-amylase and xylanase) was isolated from the cell lysate by metalaffinity chromatography using a AKTA Purifier 10 FPLC chromatograph (General Electric, USA), a 1 ml HiTrap Chelating column (General Electric, USA), activated by Ni2+ ions, and balanced with buffer containing 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, and 20 mM imidazole. The clarified supernatant was loaded onto the column and the protein was eluted in a 20-500 mM imidazole linear gradient in 20 mM Tris-HCl (pH 8.0) with 500 mM NaCl.

Birch xylan obtaining

Crushed birch sawdust was poured with distilled water at the rate of 40 ml water per 1 gram of sawdust. The resulting mixture was autoclaved for 30 minutes at a pressure of 1 atm and temperature 121°C. The supernatant was removed and alcohol-water extraction was carried out. A mixture of ethyl alcohol: water in the ratio 1:1 was prepared and boiled with a reflux condenser at 90°C for 1 hour. The supernatant was removed and alcohol-water extraction was repeatedly performed for 2 hours at 90°C. After removal of the supernatant the resulting precipitate was washed with 200 ml distilled water and dried at 105°C for 1 hour. Xylan was extracted with a solution of 4% (w/v) NaOH cooled to 18°C at a ratio of 1:40 in the solvent/dry weight ratio. The resulting suspension was shaken on a shaker at 18°C for 6 hours, the solution was passed through a filter and neutralized with acetic acid to pH 6.0, after which 90% alcohol was added in a 1:1 ratio. The filtrate was allowed to stand in the refrigerator at 4-6°C for 16 hours and centrifuged at 6000×g, for 10 minutes at 4°C. The supernatant was removed and the alcohol washing procedure was repeated twice. The washed precipitate was dried in a desiccator at 50°C. The result was a light cream-colored powder. When cold water is added, xylan swells similarly to starch and other similar polymers.

Determination of the specific activity of α-amylase and xylanase

The enzymatic activity was determined by the reducing sugars method using 3,5-dinitrosalicylic acid (DNSA) [12-14]. 1% birch xylan and potato starch were dissolved in 100 mM phosphate buffer (pH 7.0) and used as substrate. 1 mL of substrate was incubated with 40 μl of enzyme at 50°C (xylanase) and 70°C (α-amylase) for 10 minutes. The reaction was determined by adding 1.5 mL of DNSA reagent, and the mixture was boiled in a water bath for 10 min. The absorbance was measured at 540 nm on a Shimadzu UV-1900i spectrophotometer (Japan). A calibration curve was constructed using xylose and glucose (Thermo Scientific (Acros Organic), USA) as a standard. One unit of xylanase activity is defined as the amount of enzyme that releases 1 μmol of reducing sugar-xylose or glucose in 1 min under standard conditions. Measurements were performed in three three independent replicates, and the average of the three replicates was reported as the final result.

Determination of protein concentration

Protein concentration was determined according to the Bradford method [15] [200]. Bio-Rad reagent (Munich, Germany) with bovine serum albumin as a standard was used. The procedure for determining the protein concentration was performed according to the following procedure: 100 μl of Bradford reagent was mixed with 860 μl of 10% PBS and 1% glycerol and 40 μl of protein sample was added. The mixture was incubated for 2 min at room temperature and optical density was measured on a Shimadzu UV-1900i spectrophotometer (Japan) at 595 nm. The measurements were performed in three independent replicates, and the average of the three replicates was reported as the defined result.

Determination of pH and temperature optimums of enzyme action

Enzymatic activity was measured in the temperature range of 10-80°C (at 5°C intervals) in 100 mM phosphate buffer with pH 7.0 or 6.0. Enzymatic activity was measured over a pH range of 2.0 to 10.0 (at half unit intervals) at optimal tem-

perature. The maximum enzymatic activity was estimated as 100% activity; the data obtained at different pH and temperature were determined as relative activity.

Effect of metal ions on enzymatic activity

The effect of metal ions on xylanase stability of was determined in the presence of one of eight chlorides, NiCl2, MgCl2, CaCl2, CuCl2, ZnCl2, MnCl2, FeCl3, and CoCl2 at 5 mM. The enzyme was preincubated with metal chloride at room temperature for 1 h, after which activity was measured under optimal conditions.

Detection of starch hydrolysis products by TLC

Detection of hydrolysis products by TLC was performed according to [16]. For this purpose, 1% potato starch in phosphate buffer with pH 7.0 was incubated with recombinant α-amylase (10 U/ml) at 50°C for 10 hours. The reaction was stopped by heating the solution to 100°C. Samples were applied to an aluminum plate with silica gel (Silica gel 60 F254, Merck, Germany). Mono- and oligosaccharides: glucose, maltose, maltotriose, maltotetraose, and maltopentaose from Megazyme (USA) were used as standard samples. A solution of butanol, glacial acetic acid, and deionized water in a 2:1:1 (v/v) ratio was used as the mobile phase. The plates were dried at 60°C for 20 min and then placed in a developer solution containing sulfuric acid and ethyl alcohol in a ratio of 5:95 (v/v) To detect hydrolysis products, the plates were heated to 130°C until the hydrolysis products were manifested.

Detection of xylan hydrolysis products by TLC

Hydrolysis of the birch xylan products was performed according to [17]. For this purpose, 1% birch xylan in phosphate buffer with pH 7.0 was incubated with recombinant xylanase (10 U/ml) at 40°C for 10 hours. The reaction was stopped by heating the solution to 100°C. The samples were applied to an aluminum plate with silica gel (Silica gel 60 F254, Merck, Germany). Xylose, xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose (Megazyme) were used as standard samples. A solution of glacial acetic acid, chloroform, and deionized water in a ratio of 6:7:1 (v/v) was used as the mobile phase. The plates were dried at 60°C for 20 minutes and then placed in a developer solution containing sulfuric acid and ethyl alcohol in a ratio of 5:95 (v/v) and α-naphthol at a final concentration of 0.5%. For the detection of hydrolysis products, the plates were heated to 80°C until the hydrolysis products were manifested.

RESULTS AND DISCUSSION

Obtaining of recombinant α-amylase and xylanase

Transformation of *Escherichia coli* ArcticEspress(DE3) RP strain cells with pET-28c(+)/Amy and pET-28c(+)/Xyn plasmids containing α-amylase and xylanase genes resulted in strains producing recombinant α-amylase and xylanase, respectively. By culturing these strains and activating the T7 promoter, a 1-liter of recombinant culture of each strain was generated. The cell yield after induction was 5.8-6 grams for each strain. Lysates of recombinant cultures were obtained using enzymatic (using egg muramidase) and physical (ultrasonic homogenization) methods. Recombinant α-amylase and xylanase were isolated and purified from the lysates by metal affinity chromatography on Ni^{2+} ions.

Figure 1 shows the results of purification of recombinant α-amylase (Figure 1a) and recombinant xylanase (Figure 1b). As follows from the data presented, the protein eluted from the column at 150 mM imidazole and corresponds to the calculated molecular mass of recombinant α-amylase containing the hexahistidine tag, 58.8 kDa (Figure 1a). The yield of purified recombinant α-amylase is 8 mg from 1 liter of induced culture.

Previously, it was found that the optimal conditions under which recombinant α-amylase shows maximum activity are 80 °C and pH 6.0 [11]. The measurement showed that under these conditions the α-amylase activity of the enzyme was 2178 U/mg.

The calculated molecular weight of the recombinant xylanase together with the 6His-tag was 28.3 kDa, corresponding to the protein eluted at 232 mM imidazole (Figure 1b).

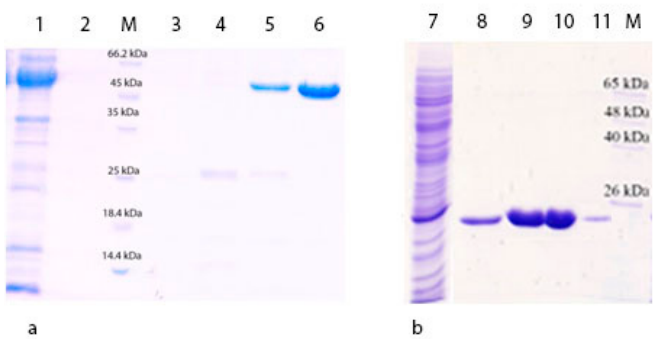

Figure 1 – Electrophoresis of the purification fractions of recombinant alpha-amylase (a) and xylanase (b): 1,7 - protein extracts of alpha-amylase and xylanase flow-throw the Ni^{2+} column, 2 - column washing with 20 mM Tris-HCl (pH 8.0) buffer, 500 mM NaCl, 20 mM imidazole, 3 , 4 - fractions with 50 and 100 mM imidazole, 5,6 - alpha-amylase fractions with 150 mM imidazole, 8 - fractions with 20 mM imidazole, 9,10 - xylanase fractions with 232 mM imidazole, 11 - fractions with 300 mM imidazole

Determination of the pH and temperature optimums of the enzyme action

The pH optimum of the recombinant xylanase action was determined to be pH 7.0 (Figure 2a), which correlates with the xylanase Xyn11 from *B. licheniformis* strain MS5-14 (pH 5.0-7.0) and Xyn10A from *Bacillus sp*. SN5 (pH 7.0) [20,20]. The recombinant xylanase retains good activity at 55% at pH 8.0, at pH below 4.0 and above 10 the enzyme loses activity. A pH-stability study of the enzyme by pre-incubating the enzymes in pH 3.0-11.0 buffers at room temperature for 10 hours demonstrates very high pH stability under both acidic and alkaline conditions. In the pH range 3-11, the enzyme retains 100% activity.

Determination of the enzymatic activity of recombinant xylanase at different temperatures (10-80 °C) showed that the temperature optimum was 55 °C (Figure 2b). This enzyme is not as thermostable as α-amylase from *B. licheniformis*. At 60 °C, the recombinant xylanase showed an activity of 86%. Above 80°C, the enzyme is completely inactivated. The more thermostable xylanases from *Bacillus subtilis* ASH and *Bacillus sp*. SV-34S have an optimum at 55 °C, as does the recombinant xylanase [22, 23]. The maximum activity of recombinant xylanase on birch xylan at the optimum temperature

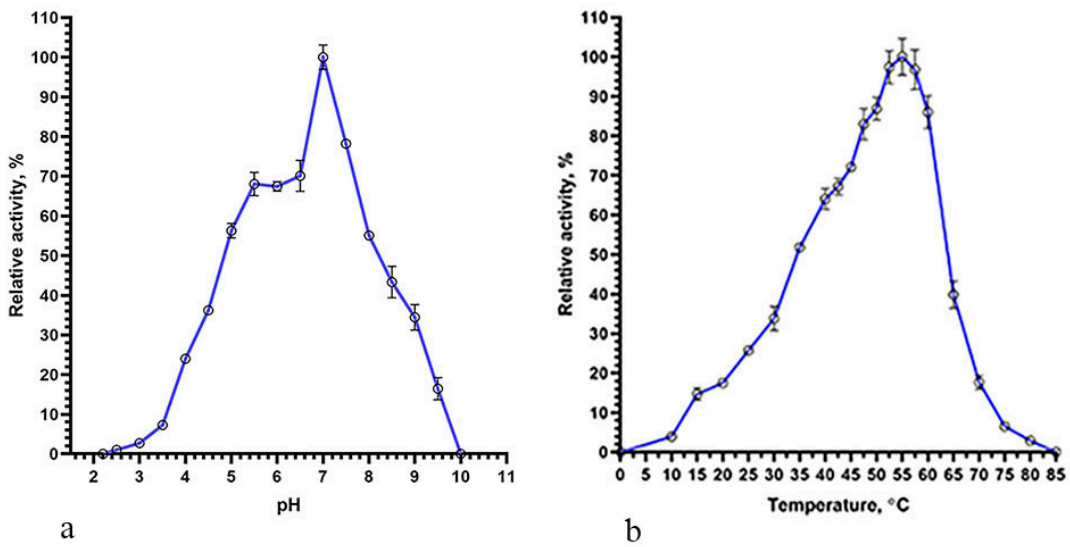

Figure 2 – Determination of pH and temperature optimums of recombinant xylanase activity

(55°C) is 1030.2 U/mg.

Effect of metal ions on enzymatic activity

Table 1 shows the effect of metal ions Ni^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Co^{2+} on xylanase activity. Ni^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{3+} ions inhibit xylanase activity by 8-26%. A special experiment on the purification of recombinant xylanase on a HiTrap Chelating 1 ml column activated with $CoCl_2$ (instead of $NiCl_2$) showed that the use of Ni^{2+} in the enzyme purification did not affect its xylanase activity. The two variants of recombinant xylanase purified on Ni^{2+} and Co2+ ions showed the same activity under the same conditions. The activity of recombinant xylanase increased by 18% after preincubation with Mn^{2+}.

Table 1 – Effect of metal ions on xylanase activity

Chemicals	Concentration	Residual activity, %
None	-	100 ± 0.9
Ni^{2+}	5 mM	73.8 ± 1.3
Mg^{2+}	5 mM	92.1 ± 2.3
Ca^{2+}	5 mM	92.3 ± 3.3
Zn^{2+}	5 mM	83.5 ± 0.5
Mn^{2+}	5 mM	118.2 ± 4.6
Co^{2+}	5 mM	101.6 ± 0.5
Cu^{2+}	5 mM	56.9 ± 3.5
Fe^{3+}	5 mM	78.4 ± 0.9

Analysis of hydrolyzed starch and xylan products by TLC

The optimal temperatures at which the studied enzymes have maximum activity are 80 °C and 55 °C for α-amylase and xylanase, respectively. However, at 80°C recombinant α-amylase from *B. licheniformis* loses more than 75% of its activity after 1 hour of incubation. On the other hand the recombinant α-amylase shows the best stability at 50°C, the enzyme fully retains its activity for 10 hours of incubation. At 50 °C, the activity of recombinant α-amylase is 51.6% of the maximum value and is 1123.8 U/mg. Recombinant xylanase from *B. sonorensis* loses more than 80% of its activity after 1 hour of incubation at 55°C. The temperature at which xylanase shows the best stability is 40°C, the enzyme fully retains its activity for 10 hours of incubation. At 40 °C, the activity of recombinant xylanase is 64% of the maximum value and is 659.3 U/mg. These temperature conditions (50 °C and 40 °C for α-amylase and xylanase, respectively) were chosen for the enzymatic hydrolysis of starch and xylan.

Detection of hydrolyzed starch and xylan products by thin-layer chromatography showed that the products mainly consist of maltooligosaccharides (Figure 3). The maltose disaccharide was found to be the dominant product. Small amounts of glucose and other oligosaccharides can be seen only after 10 hours of incubation. The obtained results show that α-amylase from *Bacillus lichenifromis* is an endoenzyme. Recombinant alpha-amylase from *Thermomyces dupontii* is known to produce mainly maltose and maltotriose and a small amount of glucose during the hydrolysis of soluble starch,

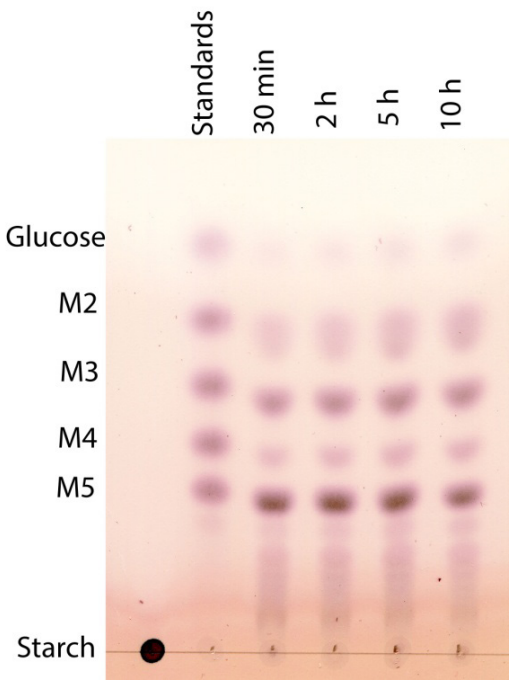

Figure 3 – Hydrolysis of potato starch using recombinant alpha-amylase

which confirms the endogenous action of the enzyme [16].

To obtain a substrate for xylanase from birch sawdust, xylan was obtained 11 grams from 200 grams of sawdust. When birchwood xylan was hydrolyzed using recombinant xylanase, the products were xylobiose and other xylooligosaccharides (Figure 4). Among the hydrolysis products, xylose was not detected even after 10 hours of enzymatic hydrolysis, indicating that, α-amylase from *Bacillus licheniformis*, and xylanase from *Bacillus sonorensis* are an endoenzyme. It was found that xylanase from the *Bacillus licheniformis* strain is also an endoenzyme in birch xylan hydrolysis; it forms xylooligosaccharides (predominantly xylothriose) and does not form xylose [24].

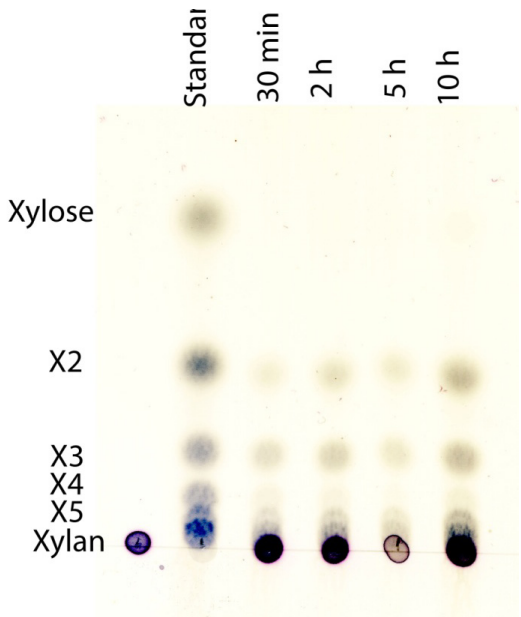

Figure 4 – Hydrolysis of birch xylan with recombinant XynT6 xylanase

CONCLUSION

Amylases and xylanases are enzymes that hydrolyze the natural polysaccharides starch and xylan. Recombinant α-amylase and xylanase were obtained using recombinant DNA technology from *Bacillus licheniformis* and *Bacillus sonorensis*, respectively. These enzymes were purified by metal affinity chromatography from the lysate of induced cultures of recombinant strains. Enzymatic hydrolysis of potato starch and birch xylan with these recombinant enzymes was performed. The hydrolysis products of these polysaccharides were studied by thin-layer chromatography.

It was found that hydrolysis products of potato starch using recombinant α-amylase were maltose, maltooligosaccharides and a small amount of glucose. Xylan was obtained from birch sawdust and hydrolyzed by recombinant xylanase. Detection of the hydrolysis products of xylan showed that xylan is hydrolyzed to oligoxylans with a degree of polymerization not less than 2 monomers. No xylose was detected among the hydrolysis products. The results indicate that α-amylase from *Bacillus licheniformis* and xylanase from *Bacillus sonorensis* are endoenzymes.

FUNDING

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ГИДРОЛИЗ РАСТИТЕЛЬНОЙ БИОМАССЫ С ИСПОЛЬЗОВАНИЕМ РЕКОМБИНАНТНОЙ АЛЬФА-АМИЛАЗЫ ИЗ *BACILLUS LICHENIFORMIS* И КСИЛАНАЗЫ ИЗ BACILLUS SONORENSIS

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АБСТРАКТ

В связи с сокращением ископаемых источников углеводородов и энергии, и увеличивающейся потребности в энергетических, химических ресурсах и продуктов питания большое значение приобретает использование биомассы в качестве возобновляемого источника. А это, в свою очередь, вызывает потребность в эффективных ферментах для биоконверсии биомассы. Ферменты имеют большое значение в промышленности, так как являются биокатализаторами сложных химических процессов. Преобразование растительной биомассы в сахара требует применения комплекса ферментов, состав которых должен быть адаптирован к типу биомассы и способу предварительной обработки. Эффективность ферментативного гидролиза можно повысить за счет оптимизации состава ферментативного комплекса, повышения каталитической активности и стабильности работы входящих в его состав ферментов. В настоящее время большое количество микробных амилаз доступно на рынке, и они почти полностью заменили химический гидролиз крахмала в перерабатывающей промышленности. Использование бактериальных ксиланаз является ключевым этапом преобразования лигноцеллюлозных полисахаридов в ферментируемые сахара для производства биотоплива и продуктов с высокой добавленной стоимостью. Ферментные комплексы, содержащие в составе и альфа-амилазы и ксиланазы, нашли применение в целлюлозно-бумажной промышленности и кормопроизводстве.

С помощью технологии рекомбинантных ДНК были получены рекомбинантные α-амилаза и ксиланаза из *Bacillus licheniformis* и *Bacillus sonorensis*, соответственно. Металлоаффинной хроматографией данные ферменты были очищены из лизата индуцированных культур рекомбинантных штаммов. Проведен ферментативный гидролиз картофельного крахмала и березового ксилана с помощью данных рекомбинантных ферментов. Методом тонкослойной хроматографии проведено исследование продуктов гидролиза данных полисахаридов. Установлено, что продуктами гидролиза картофельного крахмала с использованием рекомбинантной α-амилазы являлись мальтоза и мальтоолигосахариды и небольшое количество глюкозы. Из березовых опилок был получен ксилан, который был гидролизован рекомбинантной ксиланазой. Детекция продуктов гидролиза ксилана показала, что ксилан гидролизуется до олигоксиланов со степенью полимеризации не меньше 2. Ксилоза среди продуктов гидролиза не обнаружена. Полученные результаты свидетельствуют, что α-амилаза из *Bacillus licheniformis* и ксиланаза из *Bacillus sonorensis* являются эндоферментами.

Ключевые слова: альфа-амилаза, ксиланаза, крахмал, ксилан, *Bacillus* sp.

BACILLUS LICHENIFORMIS РЕКОМБИНАНТТЫ АЛЬФА-АМИЛАЗАСЫ МЕН *BACILLUS SONORENSIS* КСИЛАНАЗАСЫН ҚОЛДАНЫП ӨСІМДІК БИОМАССАСЫН ЫДЫРАТУ

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АБСТРАКТ

Көмірсутегі мен энергияның қазба көздерінің азаюына және энергетикалық, химиялық ресурстар мен азық-түлік өнімдеріне деген қажеттіліктің артуына байланысты биомассаны қайта өңделетін қайнар көз ретінде қолдану үлкен мағынаға ие болды. Ал бұл, өз кезегінде, биомассаның биоконверсиясы үшін тиімді ферменттерді қажет етеді. Ферменттер күрделі химиялық процестердің биокатализаторлары болғандықтан өндірісте үлкен мағынасы бар. Өсімдік биомассасын қантқа айналдыру ферменттер кешенін қолдануды қажет етеді. Олардың құрамы биомассаның түріне және алдын-ала өңдеу тәсіліне бейімделген болуы керек. Ферменттік гидролиз тиімділігін ферменттік кешен құрамын оңтайландыру, кешен құрамына кіретін ферменттердің жұмысын тұрақтандырып, каталитикалық белсенділігін көбейту арқылы өсіруге болады. Қазіргі уақытта нарықта микробтық амилазалардың көптеген түрлері бар, және де олар қайта өңдеу өнеркәсібінде крахмалдың химиялық гидролизін түгелімен дерлік алмастырды. Бактериялық ксиланазаларды қолдану лигноцеллюлозалы полисахаридтерді биоотын және жоғары қосылмалы құны бар өнімдер өндірісі үшін ферменттелетін қанттарға айналдырудың негізгі кезеңі болып табылады. Құрамында альфа-амилаза да, ксиланаза да бар ферменттік кешендер целлюлоза-қағаз өндірісінде және жем өндірісінде қолданыс тапты.

Рекомбинантты ДНҚ технологиясын пайдалана отырып, *Bacillus licheniformis* және *Bacillus sonorensis*-тен сәйкесінше рекомбинантты α-амилаза және ксиланаза алынды. Бұл ферменттер рекомбинантты штаммдардың индукцияланған дақылдарының лизаттарынан металлаффинді хроматографиясы арқылы тазартылды. Осы рекомбинантты

ферменттердің көмегімен картоп крахмалы мен қайың ксиланының ферментативті гидролизі жүргізілді. Бұл полисахаридтердің гидролиз өнімдері жұқа қабатты хроматография арқылы зерттелді. Картоп крахмалын рекомбинантты α-амилазамен ыдырату арқылы алынған гидролиз өнімдері мальтоза мен мальтолигосахаридтер және аз мөлшердегі глюкоза екені анықталды. Рекомбинантты ксиланаза арқылы гидролизденген қайың үгінділерінен ксилан алынды. Ксиланның гидролиз өнімдерін анықтау барысында, полимерлену дәрежесі кем дегенде 2 болатын, ксиланның олигоксиландарға дейін гидролизденетіні анықталды. Гидролиз өнімдерінің арасында ксилоза табылмады. Алынған нәтижелер *Bacillus licheniformis*-н α-амилазасы және *Bacillus sonorensis*-н ксиланазасы эндофермент екенін көрсетеді.

Кілтті сөздер: альфа-амилаза, ксиланаза, крахмал, ксилан, *Bacillus* sp.