# EVALUATION OF STRUCTURAL CARBOHYDRATE DEGRADING CAPACITY OF PGP BACTERIAL STRAINS USING DIFFERENT METHODS

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*Soil bacteria play an important role in carbon cycle due to their ability to decompose plant structural compounds as cellulose and hemicellulose. Structural carbohydrate degrading bacterial strains due to various metabolic activities can promote plant growth. A number of 29 plant growth promoting (PGP) bacterial strains isolated from different types of silages were tested for their cellulolytic and xylanolytic potential using two methods (dinitrosalycilic acid and p-Nytrophenyl substrate) for enzyme assay. No correlation was observed between the enzyme activities obtained for the studied bacterial strains using different methods. Due to its specificity the p-Nitrophenyl substrate method is more precise for enzyme activity determination and cellulolytic strain selection. Both cellulolytic and xylanolitic enzyme activity was observed in the case of Bacillus simplex LE101B, Paenibacillus pabuli KX101*, Paenibacillus peoriae PB2, Paenibacillus xylanexedens KC101, Arthrobacter siccitolerans KX102, Weisella paramesenteroides LC101B* and Pseudomonas rhodesiae XK2B101. The above-mentioned PGP and structural carbohydrate degrading bacterial strains have potential in conservative agriculture.*

**Keywords:** structural carbohydrate, silage bacteria, PGP bacteria, p-Nitrophenyl substrate, DNSA method

## 1. Introduction

As a result of the climate change, land degradation and biodiversity loss, soils have become very vulnerable all over the world. Soils are a major carbon reservoir containing more carbon than the atmosphere and the terrestrial vegetation combined. Soil organic carbon is part of the global carbon cycle that involves the movement of carbon through the soil, vegetation, ocean and the atmosphere. The remains of plants and animals are the main source of soil organic matter. The plants contain a variety of polysaccharides such as cellulose,

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hemicellulose and biopolymers (lignin, pectin), which are considered a renewable energy source for living organisms [1, 2].

Cellulose, as plant structural compound is a chemically homogeneous linear polymer of up to 10,000 D-glucose molecules, which are connected by β-1,4 bonds. The structural subunit of cellulose is cellobiose. Cellulose is present in pure state, in most cases and primarily associated with hemicelluloses and lignin [3].

The most abundant structural compound in nature is a hemicellulose (xylan), containing 1,4-glycosidic bonds. Xylan is present in the plant cell wall, representing 30–35% of the total dry weight. The most abundant form of xylan is heteroxylan, which comprises xylose residues in the backbone with acetyl, arabinosyl and glucuronosyl residues as substituents [4]. Hemicelluloses consist of both linear and branched heteropolymers. They mainly contain five monomeric sugars, namely D-glucose, D-mannose, D-galactose, D-xylose and L-arabinose linked together by β-1,4-glycosidic bonds. Covalent bonding between hemicelluloses and lignin provides additional strength to the plant. Xylan is the major hemicellulose of hardwoods that interacts with lignin and cellulose. Softwood hemicelluloses include: galactoglucomannans, arabinoglucuronoxylan, and arabinogalactan [5].

Hemicelluloses degrade more easily than cellulose because of low polymerization and amorphous properties. They are biodegraded to monomeric sugars and acetic acid. Hemicellulases are frequently classified according to their action on distinct substrates [6]. They complete degradation requires the cooperative action of a variety of hydrolytic enzymes like endo-1,4-β-xylanase and xylan 1,4-β-xylosidase. In addition, hemicellulose biodegradation needs accessory enzymes such as xylan esterases, ferulic and p-coumaric esterases, α-l-arabinofuranosidases, and α-4-O-methyl glucuronosidases acting synergistically to efficiently hydrolyze wood (xylans and mannans) [7, 8].

The soil microorganisms have an important role in plant biomass decomposition, because they produce the most of cellulose and hemicellulose degrading enzymes [1]. The degradation of plant cell wall polymers is made by cellulases, hemicellulases, ligninases and pectinases and auxiliary depolimerizing enzymes which fall into the following classes: glycoside hydrolases (GH), glycosyl transferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE), and auxiliary monooxygenases (AA), all enzymes have carbohydrate-binding modules [6].

Microorganisms such as aerobic bacteria (*Bacillus amyloliquefaciens*, *B. cereus*, *B. licheniformis*, *B. subtilis* and *Lactobacillus plantarum*), fungi, yeast and actinomycetes produce cellulase that degrade cellulose by hydrolysing the b-1,4-glycosidic linkages of cellulose. In contrast to aerobic bacteria, anaerobic bacteria

lack the ability to effectively penetrate into the cellulosic material which leads to the development of complex cellulase systems called cellulosome [3, 9, 10].

The carbohydrate-binding modules of cellulase enzymes contribute to cleaving the β-1,4 bonds in the cellulose chain, however hemicellulases degrade the hemicelluloses (xylans, xyloglucans, arabinoxylans, glucomannans) [1].
The cellulolysis process is controlled by the cellulase enzyme system comprising 1,4-β-endoglucanase, 1,4-β-exoglucanase and β-glucosidase. Endoglucanase randomly cleaves the β-1,4-glycosidic bonds, weather exoglucanase splits elementary fibrils from the crystalline cellulose. The third enzyme system (β-glucosidase) hydrolyses cellobiose and water-soluble cellodextrin to glucose. All three enzymes are necessarily for the complete cellulose hydrolysis [11].

Hemicelluloses are degraded by the same type of organisms as cellulose, but this degrading method requires different enzymes. Endo-1,4-β-xylanases, β-D-xylosidases, α-glucuronidases, acetylxylan esterases, and ferulic/coumaric acid esterases are obtained after complete hydrolysis of birch xylan [5].
The mycelial fungi is the major decomposer of organic matter in soil, which is composed by hydrolysis-resistant plant polymers containing cellulose. Some bacterial species can also degrade cellulose, they belong to the genera of *Firmicutes, Actinobacteria, Proteobacteria* and *Bacteroidetes* [1, 6].

Some of these structural carbohydrates degrading bacterial strains due to different metabolic activities can increase the availability of nutrients promoting the plant growth. These bacterial strains belong to plant growth promoting bacteria (PGPB) genera [12]. Plant growth-promoting (PGP) bacteria can be free-living or rhizobacteria which actively colonize plant roots, exerting beneficial effects on plant development. The PGP bacteria may promote the plant growth: using their own metabolism (solubilizing phosphates, producing hormones or fixing nitrogen) or directly affecting the plant metabolism, enhancing root development, increasing the enzymatic activity of the plant or promote the plant growth [13].

The aim of the present study was the evaluation of structural carbohydrate degrading capacity of PGP bacterial strains. Two types of analysis, a specific one using p-Nitrophenyl substrate and a non-specific one, using DNSA method were used in order to determine the structural carbohydrate degrading capacity.

## 2. Material and methods

### 2.1. Bacterial strains

Structural carbohydrate degrading bacterial strains were isolated from four different types of silages on selective agar medium. The bacterial strains were encoded according to their origin and selective media used for isolation. The first letter indicates the origin (SZ-grass, K-corn silage, L-alfalfa silage), the second

letter the used selective media (X-xylan, F-phytate, C-CMC, E-cellulose, P-pectin), and the last character indicates the number of bacterial colony. The * symbol was used for strains isolated under anaerob conditions. These bacterial strains were identified by 16S rDNA sequence analysis [14].

The used 29 bacterial strains are the following: *Bacillus aryabhattai* KF101*, *Bacillus subtilis ssp. subtilis* SZX102, *Bacillus simplex* LE101B and XK1C2101, *Bacillus subtilis ssp. inaquosorum* SZE102B*, SZX102A*, SZC102B*, SZF101B2, SZE101A and SZX101A, *Bacillus tequilensis* LE102, *Bacillus licheniformis* SZX101B*, SZC101A, SZC102A, SZE102A and SZF102, *Bacillus methylotrophicus* LC101, *Paenibacillus amylolyticus* KC102, *Paenibacillus pabuli* KX101* and KE101A, *Paenibacillus peoriae* PB2, *Paenibacillus xylanexedens* KC101, *Weissella paramesenteroides* LC101B*, *Arthrobacter nitroguaiacolicus* LX102, *Arthrobacter siccitolerans* KX102, *Paenibacillus barcinonensis* XK1A101, *Stenotrophomonas rhizophila* CK1D101, *Stenotrophomonas acidaminiphilia* CK1E101 and *Pseudomonas rhodesiae* XK2B101.

### 2.2. Analysis of structural carbohydrate degradation

#### 2.2.1. Culture condition and sampling

The bacterial strains were grown overnight in Nutrient liquid medium, 150 rpm at 28 °C. An amount of 20 mL of Bushnell Haas (BHM, $MgSO_4*7H_2O$ 0.2 g/L, $KH_2PO_4$ 1 g/L, $NH_4NO_3$ 1 g/L, $FeCl_3*6H_2O$ 0.05 g/L, $CaCl_2$ 0.02 g/L, pH=7) selective liquid medium was inoculated with 0.4 mL bacterial suspension ($OD_{595}$= 0.3) containing 5 g/L structural carbohydrate (cellulose, CMC, xylan). This step was followed by incubation at 28 °C for 14 days at 150 rpm.

At the end of the incubation period samples were taken from the culture media for both enzymatic hydrolysis and enzyme assay. For the DNSA method 1 mL bacterial suspension was obtained and centrifuged at 10000 rpm, 4 °C for 10 minutes. The supernatant was used for further analyses. For the enzyme assay, using p-Nitrophenyl substrate, 10 mL of bacterial suspension was sonicated (3x10 s, on 50 Hz and 70 amplitude) and centrifuged for 10000 rpm, at 4 °C for 10 minutes for sonicated samples. The rest of the bacterial suspension (9 mL) was centrifuged only, and further analysed for enzymatic activity.

#### 2.2.2. Estimation of enzymatic hydrolysis by dinitrosalicylic acid (DNSA) method

The amount of reducing sugar from both activated and inactivated samples was determined using DNSA method. In the case of activated samples 0.25 mL of supernatant and 0.25 mL of substrate buffer was pipetted into an Eppendorf tube and incubated for 10 min at 40 °C, followed by the addition of 1 mL of DNSA

reagent and the incubation for 10 min at 100 °C, during which colour reaction took place. Inactivated samples were obtained adding 0.25 mL of supernatant, the same amount of substrate buffer and 1 mL DNSA reagent into an Eppendorf tube, then incubated for 10 minutes at 100 °C. The samples were cooled to room temperature, centrifuged at 10000 rpm for 3 minutes. The absorbance was measured at 540 nm using ZUZI Spectrophotometer Model 4201/50. The enzyme activity was calculated using the method described by Rastogi et al. (2010) [15].

#### 2.2.3. Enzyme assay using p-Nitrophenyl substrate

*Glucosidase enzyme assay in cellulose and carboxymethyl cellulose containing broth*

In the microtiter plate were pipetted the following amounts of solutions: 50 µL supernatant, 50 µL substrate (p-Nitrophenyl β-glucopyranoside) and 100 µL phosphate buffer. For the control sample the supernatant was replaced by the same quantity of sterilized water (50 µL). The absorbance was measured at the start point on BMG LABTECH´s FLUOstar OPTIMA microplate reader at 405 nm. The microplates were incubated at 37 °C for 30 minutes, then the reaction was stopped adding 5 µL 1 M NaOH to each sample. The absorbance reading was repeated at 405 nm, and the enzyme activities were calculated following the Jackson et al. (2013) [16] protocol.

*Xylanase enzyme assay in xylan containing broth*

We used p-Nitrophenyl β-D-xylopyranoside as substrate. The enzyme assay was performed using the same protocol as described above.

## 3. Results and discussion

### 3.1. Cellulose degrading capacity of bacterial strains

#### 3.1.1. Estimation of enzymatic hydrolysis on cellulose substrate by DNSA method

In selective broth that contained cellulose, glucose production was observed in all bacterial strains. The mean value of cellulase activity varied between 0.163-0.25 U/mL (see Fig. 1.). The cellulase activity values were lower than those observed in the case of CMCase. In their study Rastogi et al. (2010) detected cellulase activity (0.03 U/mL) at the end of exponential growth phase for *Bacillus sp.* [15].

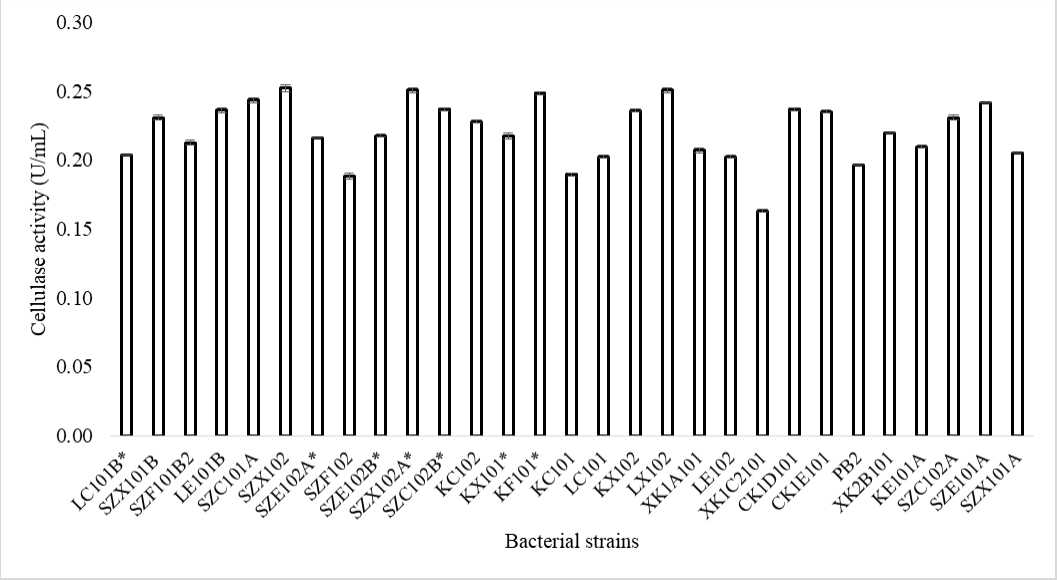


Fig. 1. Cellulase activity in a selective broth containing cellulose

### 3.1.2. Glucosidase enzyme assay in cellulose containing broth

A total number of 29 bacterial strains were tested for enzyme activity. From the selected bacterial strains 7 showed enzyme activity for glucosidase enzyme. The mean value of enzyme activity varied between 0.0012-7.51 U/mL (see Table 1.) in the case of sonicated samples, whereas the values from only centrifuged samples ranged between 0.0128-1.88 U/mL. The higher values for sonicated samples may be explained by the presence of both type of extra- and intracellular enzymes in supernatant. The lowest glucosidase enzyme activity was observed for *Bacillus licheniformis* SZX101B bacterial strain (sonicated sample). The highest glucosidase activity was showed by *Paenibacillus amylolyticus* KC102 strain in both cases (sonicated and only centrifuged samples). Seo et al. (2013) studied the β-glucosidase activity (0.63 U/mL) of *Bacillus licheniformis* using salicin (2-hydroxymethyl-phenyl-β-D-glucopyranoside) as substrate [17].

Based on literature data the *Bacillus aryabhattai*, *Bacillus licheniformis*, *Paenibacillus amylolyticus*, *Paenibacillus peoriae*, *Paenibacillus xylanexedens*, *Stenotrophomonas rhizophila*, *Bacillus subtilis*, and *Bacillus methylotrophicus* bacterial strains showed cellulase activity [17-24]. The present study is the first report of cellulase activity for the following bacterial strains: *Bacillus simplex* LE101B, *Bacillus tequilensis* LE102, *Paenibacillus pabuli* KX101* and KE101A, *Arthrobacter nitroguaiacolicus* LX102 and *Pseudomonas rhodesiae* XK2B101.

*Table 1*

**The glucosidase enzyme activity in selective broth containing cellulose**

| Bacterial strain | Glucosidase activity (U/mL) | |
|---|---|---|
| | Sonicated sample | Only centrifuged sample |
| LC101B* | 0.1785 ± 0.0474 | 0.5586 ± 0.0359 |
| SZX101B | 0.0012 ± 0.0354 | 0.0231 ± 0.0203 |
| LE101B | 0.0219 ± 0.2114 | 0 |
| SZC101A | 0.0631 ± 0.0772 | 0 |
| SZX102 | 0.0959 ± 0.0344 | 0 |
| SZE102A* | 0.1081 ± 0.0930 | 0 |
| SZF102 | 0 | 0.0971 ± 0.0264 |
| SZE102B* | 0.0631 ± 0.0369 | 0.0741 ± 0.0131 |
| SZX102A* | 0.0668 ± 0.0712 | 0 |
| KC102 | 7.5179 ± 0.7370 | 1.8871 ± 0.1309 |
| KX101* | 1.3260 ± 0.0255 | 0.6254 ± 0.0310 |
| KC101 | 0.0206 ± 0.0855 | 0 |
| LC101 | 0 | 0.0899 ± 0.0164 |
| LX102 | 0 | 1.1536 ± 0.0237 |
| XK1A101 | 0.7590 ± 0.0128 | 1.2410 ± 0.0379 |
| LE102 | 0.1761 ± 0 | 0 |
| XK1C2101 | 0 | 0.0389 ± 0.0612 |
| CK1D101 | 0.0364 ± 0.0728 | 0.2149 ± 0.0310 |
| PB2 | 0.0170 ± 0.0445 | 0.0522 ± 0.0386 |
| XK2B101 | 0.0704 ± 0.0518 | 0 |
| KE101A | 0.5695 ± 0.1392 | 0.9059 ± 0.0740 |
| SZE101A | 0.1166 ± 0.0159 | 0 |
| SZX101A | 0.0401 ± 0.1971 | 0 |

Examining the two datasets, no correlation was found between the enzyme activities obtained using the two methods (r=0.009 for centrifuged samples, r=0.035 for sonicated samples).

### 3.2. CMC degrading capacity of bacterial strains

#### 3.2.1. Estimation of enzymatic hydrolysis on CMC substrate by DNSA method

From the 29 bacterial strains selected and cultured on selective broth containing CMC, all were able to produce glucose. The mean value of CMCase (endoglucanase) activity varied between 0.16-0.42 U/mL (see Fig. 2.). The

highest amount was observed in the case of the *Stenotrophomonas acidaminiphila* CK1D101 bacterial strain. Based on the research of Gupta et al. (2012) the *Acinetobacter anitratus* and *Branhamella sp.* bacterial strains showed 0.48 U/mL, respectively 2.56 U/mL enzyme activity for CMC [11]. Mihajlovski et al. (2016) observed the highest CMCase activity (0.197 ± 0.019 U/ml) of *Paenibacillus sp.* bacterial strain on the third day of incubation [25]. The research of Moni et al. (2018) remarked CMCase activity of *Bacillus sp.* bacterial strains as varying between 0.075-0.262 U/mL [26].

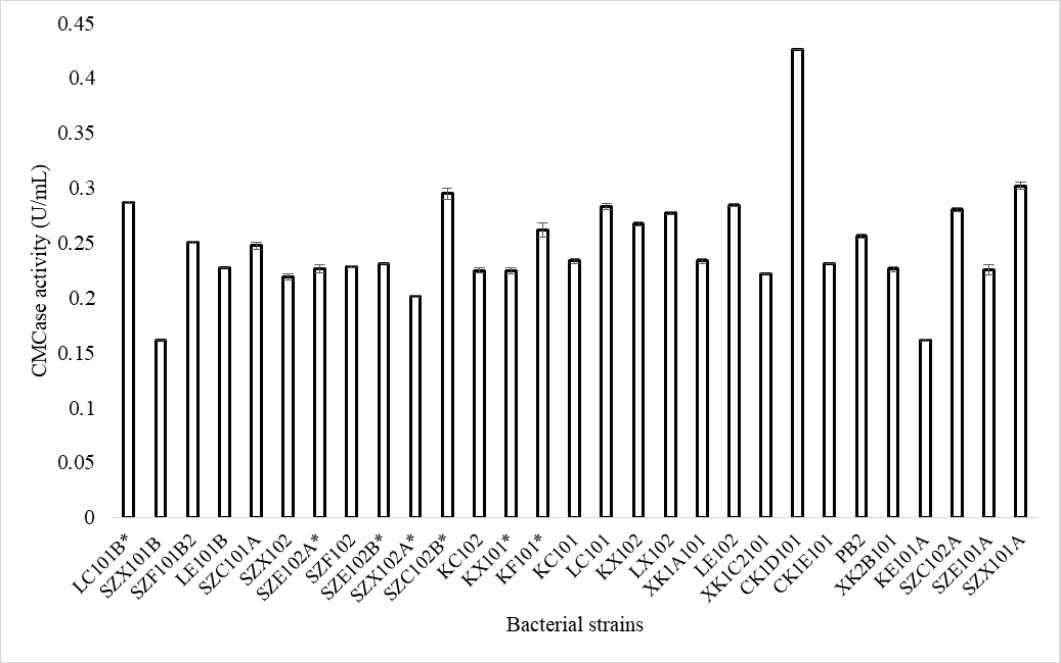


Fig. 2. CMCase activity in selective broth containing CMC

### 3.2.2. Glucosidase enzyme assay in CMC containing broth

More than 70% (21 out of 29) of the bacterial strains showed glucosidase enzyme activity (see Table. 2.). The observed values varied between 0.017-1.66 U/mL in sonicated samples and showed similar values for only centrifuged ones (0.048-1.34 U/mL). The lowest glucosidase enzyme activities were showed in sonicated samples by *Bacillus aryabhattai* KF101* and in centrifuged samples by *Bacillus subtilis subs. inaquosorum* SZE101A. *Bacillus subtilis* bacterial strain studied by Kim et al. (2012) [21] showed a much higher value of CMCase activity (1.2 U/mL) in early stage of cultivation (after 24 h). In our study *Arthrobacter nitroquaiacolicus* LX102 and *Paenibacillus pabuli* KX101* strains showed the highest glucosidase activity in centrifuged and in sonicated samples.

Scientific literature reports CMCase activity in case of *Bacillus subtilis* [21], *Bacillus licheniformis* [17], *Bacillus tequilensis* [27], *Paenibacillus amylolyticus* [25, 18], *Paenibacillus barcinonensis* [28] and *Bacillus methylotrophicus* [22]. Beside other *Bacillus sp.* and *Paenibacillus sp.* strains (*Bacillus simplex* LE101B and XK1C2101, *Paenibacillus pabuli* KX101* and KE101A, *Paenibacillus peoriae* PB2) our research reports for the first time CMCase enzyme activity for *Weissella paramesenteroides* LC101B*, *Arthrobacter nitroguaiacolicus* LX102, *Arthrobacter siccitolerans* KX102 and *Pseudomonas rhodesiae* XK2B101.

*Table 2*

**The Glucosidase enzyme activity in selective broth containing CMC**

| Bacterial strain | Glucosidase activity (U/mL) | |
|---|---|---|
| | Sonicated sample | Only centrifuged sample |
| LC101B* | 0.1044 ± 0.0111 | 0.0267 ± 0.0138 |
| SZX101B | 0.0170 ± 0.0834 | 0.0680 ± 0.0193 |
| SZF101B2 | 0.0814 ± 0.1117 | 0.1311 ± 0.0138 |
| LE101B | 0 | 0.0231 ± 0.0485 |
| SZC101A | 0 | 0.0607 ± 0.0348 |
| SZX102 | 0.0899 ± 0.0695 | 0.0801 ± 0.0438 |
| SZE102A* | 0.0631 ± 0.0765 | 0.1979 ± 0.0512 |
| SZF102 | 0.0850 ± 0.0187 | 0 |
| SZE102B* | 0.1809 ± 0.1113 | 0.0279 ± 0.0758 |
| SZX102A* | 0.1226 ± 0.1450 | 0.0413 ± 0.0421 |
| SZC102B* | 0.0850 ± 0.0327 | 0.0389 ± 0.0501 |
| KC102 | 0.3886 ± 0.0393 | 0.3084 ± 0.0895 |
| KX101* | 1.6685 ± 0.2367 | 0.7723 ± 0.0656 |
| KC101 | 0.0182 ± 0.0359 | 0.1214 ± 0.0331 |
| LC101 | 0.1858 ± 0.0602 | 0.0814 ± 0.0231 |
| KX102 | 0.1336 ± 0.0331 | 0.0826 ± 0.0690 |
| LX102 | 0 | 1.3479 ± 0.3194 |
| XK1A101 | 0.3291 ± 0.1501 | 0.2939 ± 0.0692 |
| LE102 | 0 | 0.0571 ± 0.1322 |
| CK1D101 | 0.3012 ± 0.0220 | 0.2200 ± 0.0800 |
| PB2 | 0 | 0 |
| XK2B101 | 0 | 0.0692 ± 0.0445 |
| KE101A | 0.8209 ± 0.2062 | 0.7152 ± 0.0865 |
| SZC102A | 0 | 0.0486 ± 0.0406 |

It is difficult to compare our data with others described in scientific literature, because the cellulolytic activity of the bacterial strains is influenced by the type and amount of substrate and also the growth conditions (pH and temperature) [29].

No correlation was observed between the enzyme activities obtained for the studied bacterial strains using the two methods (r=0.049 for centrifuged samples, r=0.15 for sonicated samples).

### 3.3. Xylan degrading capacity of bacterial strains

#### 3.3.1. Estimation of enzymatic hydrolysis on xylan substrate by DNSA method

The mean value of xylanase activity showed by bacterial strains in xylan containing media varied between 0.22-0.99 U/mL (see Fig. 3.).

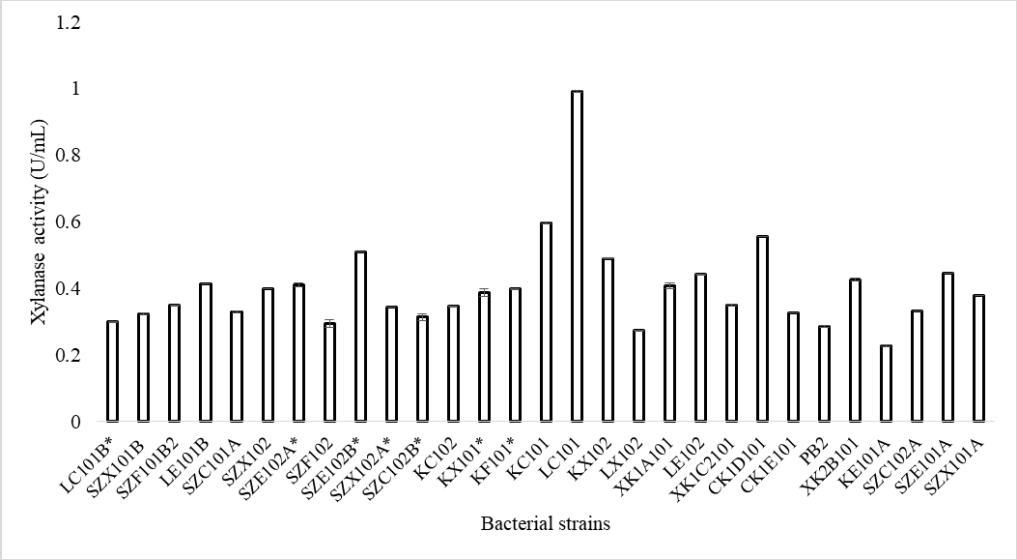


Fig. 3. Xylanase activity in a selective broth containing xylan

Two bacterial strains were outstanding, *Bacillus aryabhattai* KF101* and *Stenotrophomonas acidaminiphila* LC101, they producing the highest xylanase activity, 0.55 U/mL respectively 0.99 U/mL. After Gupta and Kar (2009) results the *Bacillus sp.* synthesized high xylanase on wheat bran medium (16.13 U/mL), but their protocol differed in the used medium (contained yeast extract peptone, YEP) and fermentation methods (SSF-solid-state fermentation, SmF-submerged fermentation) [30]. Raj et al. (2013) observed high xylanase activity values for *Stenotrophomonas maltophilia* bacterial strain on different substrates: wheat bran (26.4 ± 0.6 U/mL), birchwood xylan (23.2 ± 0.5 U/mL), rice bran (20.0 ± 0.4 U/mL) and beechwood xylan (19.2 ± 0.4 U/mL) [30]. Extremely high

values of xylanase activity were reported in a study of Subramaniyan and Prema (2000) for *Bacillus sp*. bacterial strains on 9 and 7.2 pH respectively (120 U/mL and 11.5 U/mL) [32].

### 3.3.2. Xylanase enzyme assay in xylan containing broth

From the selected 29 bacterial strains 13 showed enzyme activity for xylanase enzyme (see Table 3.).

*Table 3*

**The xylanase enzyme activity in selective broth containing xylan**

| Bacterial strain | Glucosidase activity (U/mL) | |
|---|---|---|
| | Sonicated sample | Only centrifuged sample |
| LC101B* | 0 | 0.0267 ± 0.0228 |
| SZX101B | 0.5064 ± 0.6685 | 0.1931 ± 0.0770 |
| SZF101B2 | 2.4408 ± 0.1797 | 1.0237 ± 0.0697 |
| LE101B | 0.1093 ± 0.0887 | 0.0984 ± 0.1368 |
| SZX102 | 5.6563 ± 0.1707 | 9.2107 ± 0.1339 |
| SZE102A* | 22.2380 ± 0.1719 | 21.2508 ± 0.1217 |
| SZF102 | 0.1651 ± 0.0971 | 0.1069 ± 0.0159 |
| SZE102B* | 1.6357 ± 0.0589 | 1.6636 ± 0.1794 |
| SZX102A* | 10.4942 ± 0.2435 | 9.3770 ± 0.3325 |
| SZC102B* | 0.0219 ± 0.0652 | 0.0559 ± 0.1962 |
| KC102 | 0.0984 ± 0.0187 | 0.1105 ± 0.1333 |
| KX101* | 0.2210 ± 0.0131 | 0.1384 ± 0.0553 |
| KC101 | 4.9277 ± 0.3680 | 5.6600 ± 0.2036 |
| LC101 | 0.5076 ± 0.0677 | 0.3728 ± 0.0929 |
| KX102 | 1.6308 ± 0.1675 | 1.0795 ± 0.1115 |
| XK1A101 | 0.0644 ± 0.0728 | 0.0498 ± 0.0231 |
| LE102 | 2.7055 ± 0.0566 | 1.6648 ± 0.0258 |
| PB2 | 0.0656 ± 0.0138 | 0.0194 ± 0.0222 |
| XK2B101 | 0.9314 ± 0.5190 | 1.7353 ± 0.1530 |
| SZE101A | 8.9192 ± 0.0812 | 8.5234 ± 0.2354 |
| SZX101A | 0.9654 ± 0.0278 | 1.1755 ± 0.0386 |

The mean value of enzyme activity in sonicated samples varied between 0.021-22.23 U/mL, and for only centrifuged samples the value range was between 0.0109-21.25 U/mL. The lowest xylanase enzyme activity was observed for *Bacillus subtilis ssp. inaquosorum* SZC102B* (sonicated samples) and *Bacillus simplex* XK1C2101 (only centrifuged samples) bacterial strains. The highest

xylanase activity was observed for *Bacillus licheniformis* SZE102A* in both cases (sonicated and only centrifuged samples). Hassan (2017) examined similar bacterial strains (*Bacillus sp.)* by growing the endophytic isolates on media supplemented with 1% of soluble xylan, observing a low activity of xylanase *Bacillus subtilis* strain [33]. Raj et al. (2018) observed high xylanase activity in case of *Bacillus licheniformis* strain using beechwood xylan (26.4 ± 0.5 U/mL) and birchwood xylan (23.8 ± 0.6 U/mL). In the study by Bajaj and Manhas (2012) the *Bacillus licheniformis* bacterial strain produced the highest amount of xylanase activity (30 U/mL) when xylan was used as carbon source, whereas the xylanase activity observed was lower when wheat bran (28 U/mL) and rice bran (26 U/mL) substrates were used [35].

Xylanase activity was detected earlier for *Bacillus aryabhattai*, *Bacillus subtilis* [21], *Bacillus licheniformis* [17], *Bacillus tequilensis* [36], *Paenibacillus amylolyticus* [18], *Bacillus methylotrophicus* [22], *Paenibacillus xylanexedens* [20], *and Paenibacillus barcinonensis* [37] bacterial strains. No evidence of xylanase activity for *Bacillus simplex*, *Paenibacillus pabuli*, *Paenibacillus peoriae*, *Arthrobacter siccitolerans* and *Pseudomonas rhodesiae* was found in the scientific literature, therefore this is the first study highlighting this.

We compared the xylanase enzyme activities obtained with different methods, but no correlation was found (r=0.086 for centrifuged and r=0.078 for sonicated samples).

## 4. Conclusions

The structural carbohydrate degrading capacity using dinitrosalicylic acid method was the highest in the case of *Bacillus subtilis ssp. inaquosorum* SZX102A* on cellulose substrate, *Bacillus methylotrophicus* LC101 on xylan substrate and *Stenotrophomonas acidaminiphila* CK1D101 on CMC substrate.

*Paenibacillus amylolyticus* KC102 (cellulose substrate), *Paenibacillus pabuli* KX101* (CMC substrate, sonicated samples) and *Arthrobacter nitroquaiacolicus* LX102 (CMC substrate, only centrifuged samples) showed the highest glucosidase activity, while the highest xylanase activity was observed in the case of *Bacillus licheniformis* SZE102A* (xylane substrate).

No correlation was observed between the enzyme activities obtained using different methods. Due to the non-specificity of DNSA method the specific method using p-Nitrophenyl substrate is more precise for enzyme activity determination and cellulolytic strain selection.

Co-production of cellulolytic and xylanolitic enzymes was observed in the case of *Bacillus simplex* LE101B, *Paenibacillus pabuli* KX101*, *Paenibacillus peoriae* PB2, *Paenibacillus xylanexedens* KC101, *Arthrobacter siccitolerans* KX102, *Weisella paramesenteroides* LC101B* and *Pseudomonas rhodesiae*

XK2B101. The above-mentioned PGP bacterial strains are capable of cellulosic biomass decomposition, having potential in conservative agriculture.

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