

## Isolation of Thermophilic Enzyme-producing *Parageobacillus* Bacteria from Chipped Woody Waste

Olesya Yunitsyna,<sup>a</sup> Igor Sinelnikov,<sup>a</sup> Oksana Kisil,<sup>a</sup> Ksenia Bolotova,<sup>a</sup> Andrey Aksenov,<sup>a</sup> and Galina Simonsen<sup>b</sup>

The morphological and biochemical properties were investigated for 18 types of thermophilic bacteria isolated from a woody-chip pile at a wood processing plant in Northern Russia. Genetic fingerprinting and 16S rRNA identification were used to divide the investigated microorganisms into groups according to their genetic affiliation. It was found that the isolated bacteria belonged to a minimally studied genus of *Parageobacillus* and exhibited optimum temperature and pH in the ranges of 57 to 60 °C and 7.0 to 8.5, respectively. The amylase activity was determined for all of the 18 isolated strains. Catalytic properties of the bacteria-produced xylanases were evaluated with respect to their activity towards xylan and xylan-containing carbon substrates. Biotechnological potential of the two most promising bacterial strains (*Parageobacillus caldoxylosilyticus* and *Parageobacillus thermoglucosidasius*) and their possible use in xylanase production was evaluated. The results showed that bacteria present in the chipped woody waste is an important source of thermoalkalophilic enzymes.

**Keywords:** *Parageobacillus caldoxylosilyticus*; *Parageobacillus thermoglucosidasius*; Woody-chip pile; Thermophiles; Amylases; Xylanases

**Contact information:** a: Department of Biology, Ecology and Biotechnology, Northern (Arctic) Federal University, Arkhangelsk, Russia; b: Department of Chemical Engineering, Norwegian University of Science and Technology (NTNU), Trondheim, Norway; \*Corresponding author: galinanorge@gmail.com

### INTRODUCTION

Modern industrial biotechnology demands the development of high-performance biocatalysts, or enzymes, applicable in a variety of industrial operations (Choi *et al.* 2015; Jemli *et al.* 2016; Blamey *et al.* 2017; Madhavan *et al.* 2017). Enzyme-assisted processes are of great importance in waste minimization and recycling due to the high efficiency of enzymes, their low consumption, and selectivity in removal of unwanted compounds. Cellulases constitute a well-known class of enzymes capable of hydrolyzing 1,4- $\beta$ -D-glucosidic linkages in cellulose, which is a polymer available globally for bioconversion into numerous products. Cellulases have a long history of applications, including processing of wood biomass, textile and laundry, food, and bioethanol production (Coughlan 1985; Hardiman *et al.* 2010; Behera *et al.* 2017). In addition to cellulases, other enzymes capable of hydrolyzing non-cellulosic polysaccharides, such as amylases and xylanases (Hu *et al.* 2015), are continuously gaining importance in the global market of bioconversion and recycling processes (Pandey *et al.* 2000; Saxena and Singh Chauhan 2016). Amylases is a group of enzymes capable of hydrolyzing glycosidic bonds in starch, namely,  $\alpha$ -amylase,  $\beta$ -amylase, and glucoamylase. The amylases are traditionally used at elevated temperatures and can be derived from a variety of plant and microbial sources (Gopinath *et al.* 2017). They are commonly utilized in the processing of raw grain materials (Nigam and Singh 1995), removal of starch present in waste paper and paperboard (Saxena and Singh Chauhan 2016), and paper recycling (Raul *et al.* 2014). The hydrolysis of xylan,

a major component of hemicelluloses, can be achieved by xylanases or xylanase mixtures containing endo- $\beta$ -1,4-xylanases and  $\beta$ -D-xylosidases (Polizeli *et al.* 2005). Xylanases have been used by the industry since the early 1990's. They are recognized for their catalytic effects in improving efficiency of the existing pulp bleaching processes (Viikari *et al.* 1994) and bioconversion of agricultural waste into fuel and chemicals (Nigam and Pandey 2009). One of the main challenges in xylanase production from bacterial sources is the high cost of the xylanase inducer, pure xylan (Alves *et al.* 2016). Waste materials from wood processing plants, as well as other lignocellulosic sources, can serve as alternative carbon substrates for the production of xylanases. The use of such substrates can reduce the cost of the entire process. At present, researchers are looking for novel bacterial strains capable of assimilating lignocellulosic waste as a cost-effective carbon source for the production of xylanases (Bhalla *et al.* 2015).

One of the important features of microbial enzymes is that they exhibit substrate selectivity and stability under abnormal conditions, mainly high temperatures and pH extremes. Based on their stability, certain enzymes can be categorized as thermophilic, acidophilic, or alkalophilic. These properties make them valuable contributors to multiple industrial processes. One example is the application of thermoalkalophilic enzymes in pulp and paper production and paper recycling, where both the temperatures and pH often rise above 50 °C and 8, respectively. The thermoacidophilic enzymes can contribute to enhancing mass transfer and reducing substrate viscosity during hydrolysis of raw plant materials in industrial processes (Cai *et al.* 2011).

Microbial enzymes can be produced by different microorganisms, both bacterial and fungal species. Although fungal species are significant producers of thermostable enzymes, the obtained enzymes often lack stability at high temperatures and pH and do not qualify for use in certain biotechnological applications (Kumar *et al.* 2013). Therefore, the search for new bacteria that are capable of synthesizing stable enzymes is highly relevant. The enzyme-producing bacteria can be isolated from a variety of waste sources, one of which is the chipped woody waste produced in abundance by wood processing plants. The composition, and therefore bacterial presence in such wastes, will vary depending on the season, storage, mixing conditions, type of wood waste, and many other factors. This variety gives endless possibilities for discovering and extracting new bacterial species with unique properties that have not been previously reported.

In this work, several types of bacteria have been isolated from samples of a chipped woody waste pile located at a wood processing plant in Northern Russia. The bacteria are identified and characterized with respect to their amyolytic and xylanolytic properties. Two bacterial strains, *P. caldoxylosilyticus* and *P. thermoglucosidasius*, were chosen for detailed study due to their promising ability to produce xylanolytic enzymes. A selection of carbon substrates was used to evaluate the bacterial performance in terms of xylanase activity and xylanase production in industrial conditions.

## EXPERIMENTAL

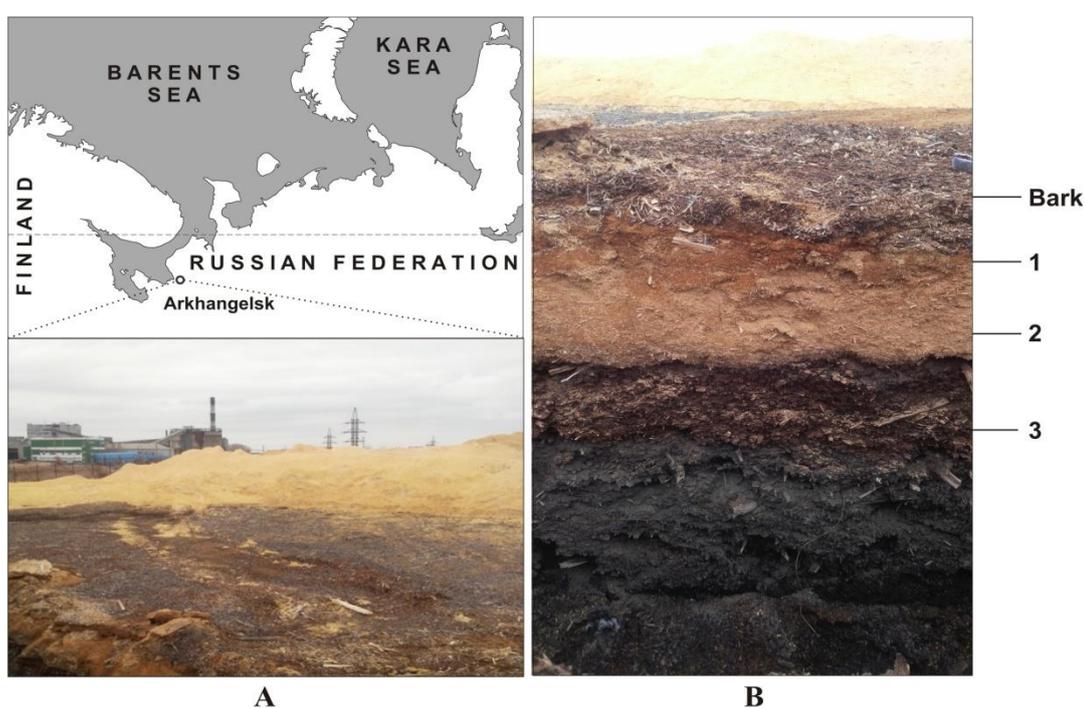
### Materials

#### *Study site, sampling, and bacteria isolation*

Samples of wood chips were collected from a woody chip pile at a timber mill named Solombalales located in the city of Arkhangelsk in Northern Russia (64°35'19"N, 40°33'20"E) (Fig. 1A). The sampling was carried out at the following depths: pile surface (bark), 2 to 10 cm, 10 to 30 cm, and 80 to 100 cm (Fig. 1B). The pile was semi-anaerobic

at each sampling point due to periodic mixing. The samples were placed into sterile plastic containers (500 mL) and transported to the laboratory at ambient temperature.

Once the samples arrived, they were sealed separately into 100-mL airtight bottles containing 0.1% peptone solution and kept at 65 °C for five days stirring at 150 rpm. The primary screening of the bacteria was carried out in a minimal medium (MM) containing potassium hydrogen phosphate trihydrate ( $K_2HPO_4 \cdot 3H_2O$ ; 5.24 g/L), magnesium sulfate heptahydrate ( $MgSO_4 \cdot 7H_2O$ ; 1 g/L), potassium chloride (KCl; 0.2 g/L), iron(II) sulfate ( $FeSO_4$ ; 11.4 mg/L), xylan (1%), peptone (0.5%), yeast extract (0.1%), agar (1.5%), and trace element solution (1 mL). The system was incubated for 24 h at 60 °C (Valladares Juárez *et al.* 2009). Cultures showing different colony morphology were separated and further purified by streaking on the same medium at least three more times. The purified bacteria cultures were stored in glycerol stock (15%) at -80 °C.



**Fig. 1.** Woody-chip pile located at the timber mill Solombalalets (A - study site) and sampling points (B)

## Methods

### *Morphological and biochemical characterization of the isolated bacterial strains*

The morphology and visual appearance of the bacterial strains was evaluated by scanning electron microscopy (SEM) using a SIGMA VP microscope (Carl Zeiss, Oberkochen, Germany) at an accelerating voltage of 10 kV with an in-lens detector. The strains were cultivated for 24 h at 55 °C on a Luria-Bertani (LB) medium. A piece of the medium was separated and freeze-dried using a FreeZone 2.5 L Benchtop Freeze Dry System (Labconco). The dried samples were then used for the microscopic analysis. The spore formation was monitored with a Primo Star (Carl Zeiss, Oberkochen, Germany) optical microscope. Single bacterial colonies were observed on agar plates at different incubation periods.

The Gram type of the isolated strains was determined using a Gram coloring kit in accordance with the manufacturer's instructions (JSC "Ecolab", Moscow, Russia).

The utilization of carbon compounds was determined using differential diagnostic media of Hiss (Sinelnikov *et al.* 2017) including one of the carbon sources (glucose, lactose, maltose, mannitol, sucrose, or sorbitol). A positive result was indicated by a change in medium color after cultivation of the isolates for 18 to 24 h at 60 °C.

#### *Polymerase chain reaction (PCR) fingerprinting with (GTG)<sub>5</sub> primer*

The repetitive sequence-based (GTG)<sub>5</sub>-PCR fingerprinting methods were only used for preliminary genotypic characterization of bacteria to avoid high costs associated with comparative characterization of the species. The sequence (5'-GTGGTGGTG-GTGGTG-3') was used as a primer (Rasschaert *et al.* 2005). The PCR reaction was activated in a 25 µL solution containing 25 pmol of primer, 2.5 µL 10 × PCR buffer (10 mM Tris, 50 mM KCl, pH 8.3), 200 µM of deoxynucleotide (dNTPS), 25 mM of magnesium chloride (MgCl<sub>2</sub>), 1 U of Taq DNA polymerase, and 4 µL of template DNA. The cycling conditions were the same as for the (GTG)<sub>5</sub>. The initial denaturation was performed at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 8 min. Approximately 8 µL of the PCR product was loaded into 1% agarose gel and followed by the fingerprinting analysis (Kathleen *et al.* 2014).

#### *16S rRNA gene sequence analysis*

Single bacterial colonies were picked out and introduced into a pre-warmed LB medium. A total of 2 µL was transferred into a PCR tube after cultures reached the exponential phase. The measured sample absorbance at a wavelength of 600 nm (OD<sub>600</sub>) was between 0.5 and 1. The PCR mix containing 1.25 units of DreamTaq DNA polymerase (Alkor-Bio, Saint Petersburg, Russia), 10 × of DreamTaq buffer (Alkor-Bio, Saint Petersburg, Russia), 100 µM of dNTPs (Alkor-Bio, Saint Petersburg, Russia), 0.2 µM of both 27F and 1492R primers, and Milli-Q water was added to a total volume of 50 µL (Daas *et al.* 2016). The PCR conditions were as follows: initial denaturation at 95 °C for 10 min, 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 1.5 min with the final extension at 72 °C. The PCR amplification was performed with a T100™ Thermal Cycler (Bio-Rad, Hercules, USA). The amplified products were purified, and the sequence of the 16S rRNA gene was determined by DNA sequencing with the Sanger method on a DNA sequencer NANOFORM-05 (Synthol, Moscow, Russia). The sequence database comparison was performed using the BLAST software through the National Center for Biotechnology Information (NCBI, Bethesda, Maryland) website.

#### *Plate test for amylase activity*

The isolated bacterial strains were screened for amylase activity by a starch hydrolysis test. The purified strains were grown on the agar MM medium containing 1% soluble starch at 60 °C for 24 h. The presence of amylase activity was confirmed by the appearance of clear halos around colonies after staining with Lugol's iodine (Teodoro and Martins 2000).

#### *Xylanase production and assay of xylanase activity*

The xylanase activity was determined by testing extracts of the isolated bacterial strains. The cells were cultivated in the liquid MM medium with 1% oat spelt xylan at 60 °C for 24 h. The culture fluids were centrifuged at 4200 rpm for 20 min at 4 °C. The xylanase activity was measured using the Nelson–Somogyi method (Sinitsyna *et al.* 2003). The reaction mixture contained 40 µL of appropriately diluted culture filtrate and 160 µL

of the 1% xylan solution that was prepared in the acetate buffer (0.05 M, pH 5.0) and then incubated for 10 min at 50 °C. After incubation, the reaction was stopped by the addition of 200 µL of Somogyi reagent and heated for 40 min in a hot water bath. After cooling, 200 µL of Nelson reagent (Helicon, Saint Petersburg, Russia) was added. After 15 min, 400 µL of acetone and 1000 µL of Milli-Q water was added. The resulting reducing sugars were measured spectrophotometrically (IMPLEN, München, Germany) at 610 nm and expressed as xylose equivalent. Xylose was taken as a standard (Sinelnikov *et al.* 2017). One-unit enzyme activity was defined as the amount of enzyme required to produce 1 mmol reducing sugar per min under standard assay conditions.

#### *Effect of substrate specificity on xylanase activity*

Three different carbon substrates, birch chips, brewers' spent grain, and oat spelt xylan, were used to test the effect of different carbon sources on the xylanase activity. The bacteria were cultivated in a liquid medium containing K<sub>2</sub>HPO<sub>4</sub> (2.5 g/L), KH<sub>2</sub>PO<sub>4</sub> (0.5 g/L), CaCl<sub>2</sub> (0.2 g/L), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.5 g/L), peptone (0.5%), yeast extract (0.2%), glucose (20 g/L), and 1% (w/v) of one of the carbon substrates (Alves *et al.* 2016). The cell culture fluids were used as the enzyme source. The xylanase activity was measured using the Nelson-Somogyi method.

#### *Optimal conditions for bacterial growth*

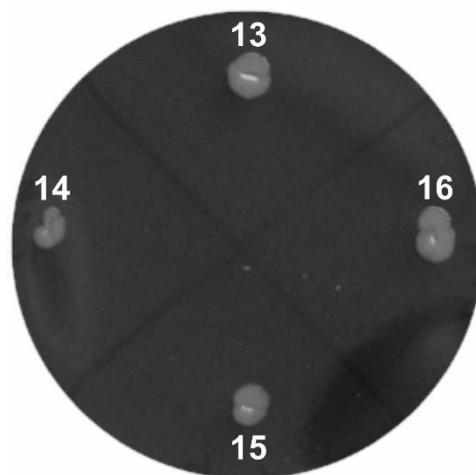
The optimal temperature range for bacterial growth was determined after incubation in the LB liquid medium at a temperature range from 55 °C to 70 °C. The pH influence on bacterial growth was tested after incubation in the LB liquid medium pH, which ranged from 5 to 11. The optimum growth was established by measuring an increase in turbidity at the wavelength of 600 nm. The turbidity tests were performed using a NanoPhotometer NP80 spectrophotometer (IMPLEN, München, Germany).

## RESULTS AND DISCUSSION

Large quantities of chipped woody waste deposits are produced and stored in Northern parts of Russia due to a large and ever-growing timber industry. The deposits are affected by relatively high temperatures in the deep layers during summer periods, which makes them a suitable and unique place for the development of thermophilic microorganisms. The research focusing on the isolation, identification, and characterization of enzymes produced by thermophilic microorganisms typically includes a preliminary study of the hydrothermal sources to find out whether or not they contain any unique microorganisms. Their isolation from self-heating wood waste deposits inherently introduces a high potential of obtaining industrially important enzymes applicable in the bioconversion of plant materials. Larsen and McCartney (2000) reported that composting deposits of different types of bark and wood chips could contain as much as 20 to 35% of free air space throughout the composting process. This ensures easy access of oxygen to the upper layers of the pile and allows for growth of the thermophilic microorganisms (Handelsman 2004; Streit *et al.* 2004).

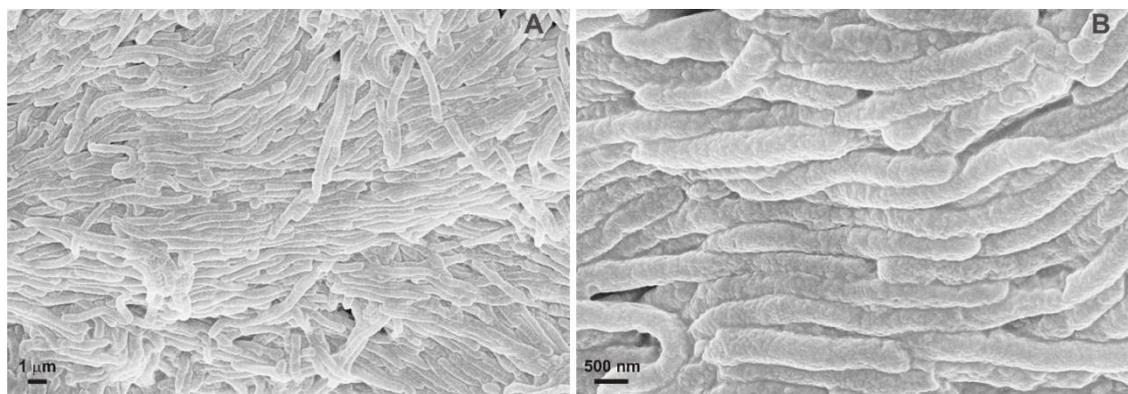
In the current work, a total of 18 bacterial strains were isolated from a woody-chip pile sample during a screening process. The morphological characterization was performed based on visual appearances of the bacterial colonies as well as SEM images of the separate cells. The isolated strains differed slightly according to their morphological features. It was observed that smooth and round colonies of beige and light-brown colors were formed on

a solid medium. Examples of bacterial colonies grown for 24 h from strains 13 to 16 are presented in Fig. 2.



**Fig. 2.** A photograph of bacterial colonies on agar medium (strains 13 to 16)

All of the bacterial isolates were moderately thermophilic, aerobic or facultative anaerobic, motile, and Gram-positive rods. The isolates were observed as single cells, 4- to 5- $\mu\text{m}$ -long and 1.0- to 1.5- $\mu\text{m}$ -wide (Figs. 3 A and 3B). Figures 3A and 3B are SEM images that show a fully-grown bacterial colony (after 24 h) under different magnifications. The cells were arranged into long chains and showed signs of spore formation.



**Fig. 3.** SEM images of bacteria isolated from woody-chip pile

The biochemical characterization tests were performed on the Hiss's media and revealed no noticeable differences in the metabolism of the studied bacterial strains. All of the isolates showed high utilization of various carbon sources with varying ability to utilize sucrose, glucose, and maltose (Table 1). The broadest spectrum of carbohydrates was utilized by bacterial strains isolated from the bark layer (strains 13 to 18) and from relatively deep layers of the woody-chip pile (strains 10 to 12).

Identification techniques based on phenotype usually show discrepancies between phenotypic and genotypic identifications (Renneberg *et al.* 1995). It is known that gene sequencing using gold as a standard provides the most accurate genotypic identification of species. However, gene-sequencing methods may be economically unviable in analyses of large amounts of isolates. DNA fingerprinting is based on a PCR amplification of genomic

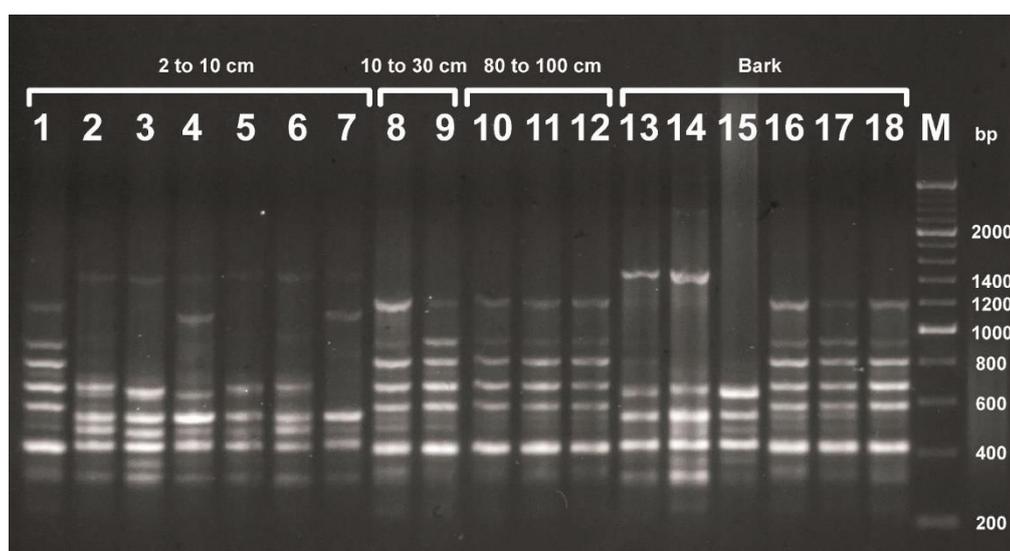
elements between interspersed repeated DNA sequences. This results in a specific DNA band pattern after (GTG)<sub>5</sub>-PCR fingerprinting.

**Table 1.** Summary of Carbohydrate Utilization by Isolated Bacterial Strains

Strain	Glucose	Lactose	Sucrose	Sorbitol	Maltose	Mannitol	Xylose
1	+	+	-	+	+	+	+
2	+	+	-	+	+	±	+
3	-	+	-	+	-	±	+
4	+	±	-	+	-	+	+
5	-	+	±	+	+	±	+
6	+	±	-	+	+	±	+
7	+	+	-	+	-	±	+
8	+	+	±	+	±	+	+
9	±	+	±	+	±	+	+
10	+	+	±	+	+	+	+
11	+	+	±	+	+	±	+
12	+	+	±	+	+	+	+
13	+	±	-	+	+	±	+
14	+	±	-	+	±	±	+
15	+	±	-	+	±	+	+
16	+	±	±	+	+	±	+
17	+	+	-	+	+	+	+
18	+	+	+	+	+	±	+

«+» positive, «-» negative, «±» variable sign

In this study, the repetitive sequence-based (rep-PCR) technique was successfully applied for the comparative identification of the 18 different bacterial strains. The molecular typing consisted of 5 to 9 fingerprint bands ranging in size from 0.2 Kb to 1.4 Kb. Several distinct polymorphic bands were observed for all of the bacterial strains, and the results are presented in Fig. 4.

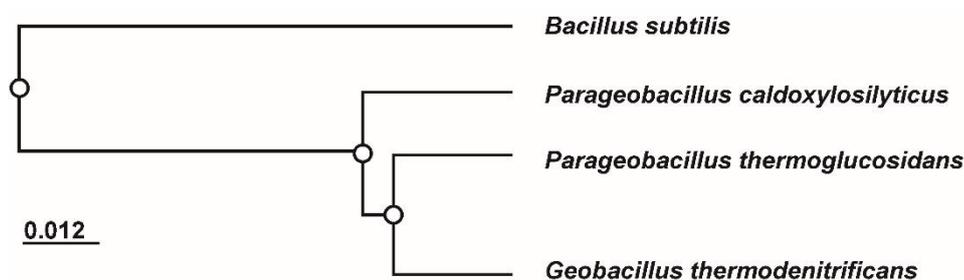


**Fig. 4.** PCR amplification of (GTG)<sub>5</sub> DNA fingerprinting (lanes 1 to 18 represent bacterial strains, lane M is 200 bp DNA Ladder)

The (GTG)<sub>5</sub> band pattern revealed similar genetic fingerprints for all four sampling points. A 100% match of profiles was detected within several groups (sampling points of

10 to 30 cm and 80 to 100 cm). The above procedure gave a rough estimate, based on the sampling locations, of genetic similarity among the bacterial strains.

The species identification of the bacterial strains was conducted by the 16S rRNA sequence analysis, a standard technique in bacterial taxonomy. It is also commonly used in the 'polyphasic approach,' when new descriptions of microbial species, or higher taxa, are written (Ludwig and Schleifer 1999). There are some limitations when comparing the 16S rRNA gene sequences of phylogenetically homogeneous bacterial groups because the structurally conserved sequences found in the 16S rRNA gene might not allow strains to identify up to the species level in closely related microorganisms. Moreover, it was assumed that species with 70% or greater DNA similarity usually have more than 97% sequence identity. This made it possible, with a high probability, to relate the isolated strains to thermophilic bacteria. As a result of the 16S rRNA gene sequencing, the isolated strains were identified as *Parageobacillus caldoxylosilyticus* and *Parageobacillus thermoglucosidans*. Phylogenetic tree illustrating the evolutionary history of bacteria is presented in Fig.5.

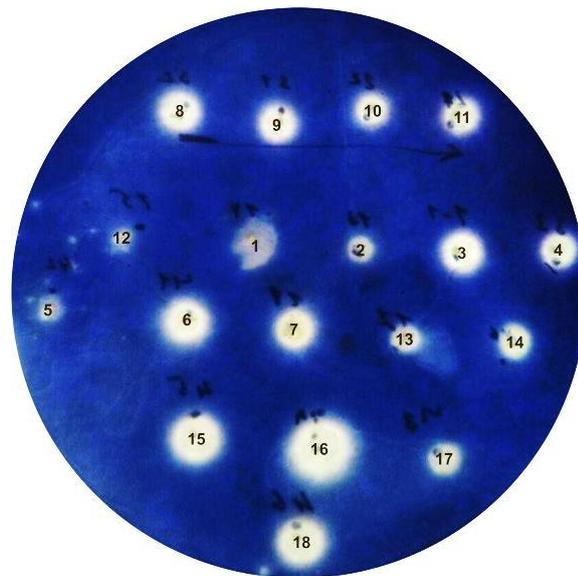


**Fig. 5.** Phylogenetic tree illustrating the evolutionary history of bacteria, constructed using the maximum composite likelihood function (evolutionary history) and neighbor-joining method within the program MEGA

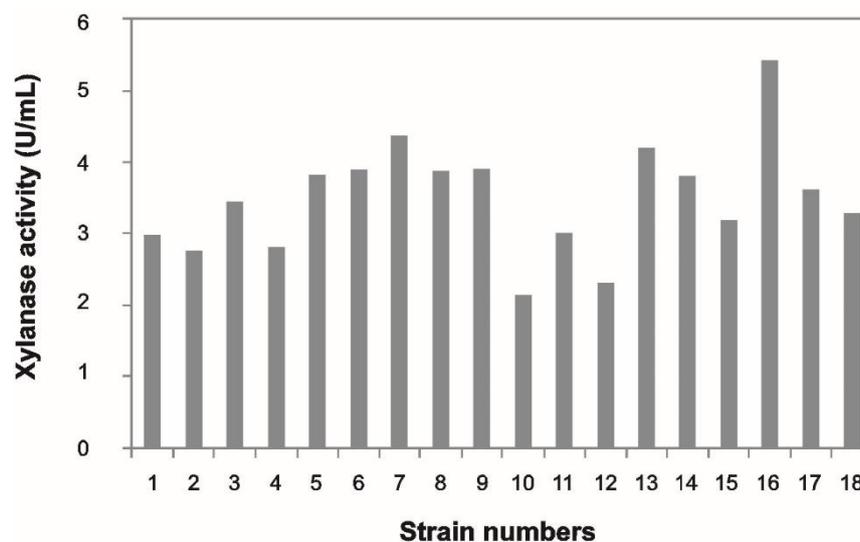
The abovementioned genus of *Parageobacillus* was classified as a *Geobacillus* genus, isolated from petroleum reservoir formation waters and first described back in 2001 (Nazina *et al.* 2001). Fifteen years later the *Geobacillus* genus was re-assessed and divided into the genus of two separate genera, *Geobacillus* and *Parageobacillus*, differing in terms of their nucleotide base compositions (Aliyu *et al.* 2016). Today, the *Parageobacillus* species and their secretome remain largely unexplored. The few available studies suggest possible use of the species in bioethanol and hydrogen production (Bibra *et al.* 2017; Mohr *et al.* 2018). The bacterial strains were also reported to produce mixtures of organic acids, proteins, enzymes, and toxins (Bibra *et al.* 2017; Lebre *et al.* 2018).

The 18 isolated bacterial strains were examined with respect to their amylase activity on agar plates with the addition of 1% starch. The formation of clearing zones around each colony indicated the presence of amylase activity as a characteristic feature of all the studied strains (Fig. 6). The maximum amylase activity was observed in strains 15, 16, and 18, which had been isolated from the bark layer of the woody-chip pile. A possible explanation could be that the processed trees were obtained in the period from July to October, when most of the starch accumulates in the tissues of the bark (Yoshioka *et al.* 1988).

Testing the xylanase activity was another important task performed with the aim of defining the most promising producers of xylanolytic enzymes. A quantitative comparison was conducted for the enzyme complexes isolated from culture fluids of the strains. The values were calculated based on the initial rates of xylan hydrolysis.



**Fig. 6.** The photograph of 18 bacterial strains subjected to the amylase activity tests

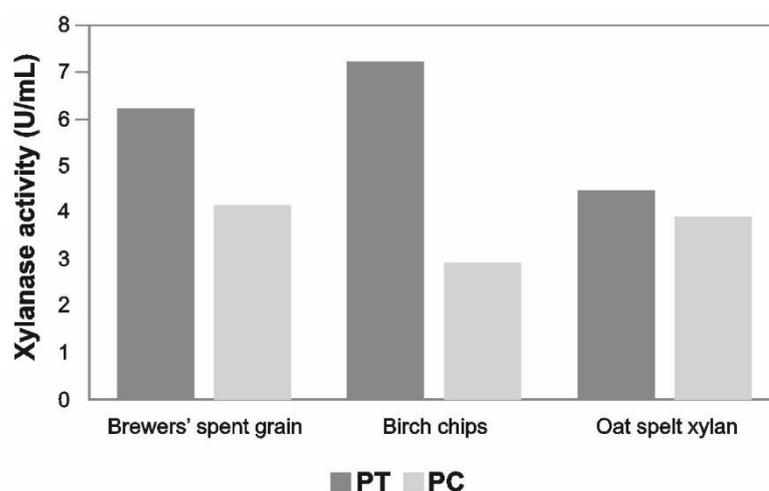


**Fig. 7.** Xylanase activities of 18 bacterial strains

Figure 7 shows that all of the isolates were xylanase positive and xylanase activity ranged from 2.1 to 5.4 U/mL. Strains 7 and 16 showed the highest values of xylanase activity and were selected for further experiments. It should be mentioned that the extracellular activity levels in all the 18 strains were quite low compared to other strains reported in the literature (Bibi *et al.* 2014; Thebti *et al.* 2016). This could have been due to characteristics of the habitat, where secretion of the extracellular enzymes in large quantities can be too energy consuming for a microorganism. It was concluded that practical utilization of the *P. caldxylosilyticus* and *P. thermoglucosidasius* strains for xylanase production will require genetic improvement including the removal of genes responsible for the release of undesirable compounds.

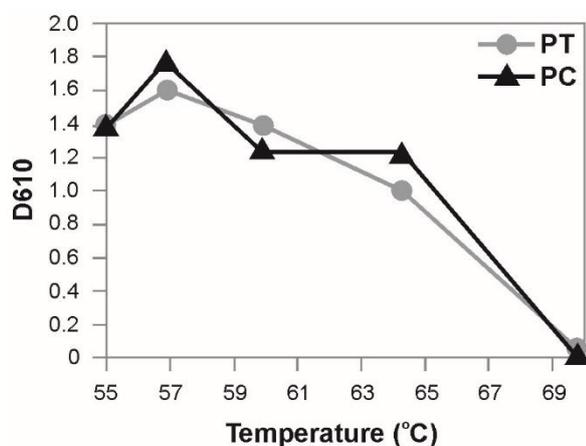
The substrate specificity experiments were performed using birch chips, brewers' spent grain, and oat spelt xylan. The activities of the xylanases synthesized by *P. caldxylosilyticus* and *P. thermoglucosidasius* were compared depending on the substrate

origin. The bacteria were grown in a culture medium containing each substrate for 24 h at 60 °C. It was determined that all of the selected substrates were capable of supporting bacterial growth accompanied by the production of xylanases. The results are presented in Fig. 8. The birch chips and brewers' spent grain were the best carbon sources for xylanase synthesis by *P. thermoglucosidasius* (PT), attaining 7.23 U/mL and 6.25 U/mL, respectively. The maximum xylanase activity secreted by *P. caldxylosilyticus* (PC) was achieved in the medium containing brewers' spent grain (4.15 U/mL). It was followed by oat spelt xylan (3.90 U/mL) and birch chips (2.91 U/mL). The variation in xylanase activity, depending on the type of substrate, may have been due to the fact that the materials contain xylan of different compositions. As a result, the bacterial strains synthesize xylanases specifically for a particular kind of bond. It can also be that the carbon substrates contain lignin, which is capable of inhibiting the activity of xylanases (Berlin *et al.* 2006).

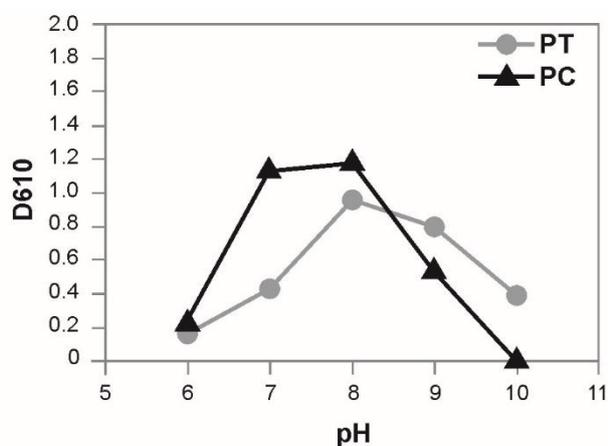


**Fig. 8.** The effect of carbon substrates on the xylanase activity of PC and PT strains

Bacteria live in and adapt to a wide diversity of environmental conditions. Certain factors can promote growth, while others will slow it down. Determination of the optimal growth conditions for the bacterial strains is an important step in achieving the best possible bacterial performance. The effects of temperature and pH on the growth of the two chosen bacterial strains are shown in Figs. 9 and 10.



**Fig. 9.** The effect of temperature on the growth of PC and PT



**Fig.10.** The effect of growth medium pH on the growth of PC and PT

The values were plotted against the optical density measured at a wavelength of 610 nm. The optimal temperature and pH for the growth of the *P. thermoglucosidasius* was established as 57 to 60 °C and 8.5, respectively. The optimal temperature and pH for the growth of the *P. caldxylosilyticus* was 57 to 60 °C and 7, respectively. The bacterial growth slowed down at temperatures below 55 °C and above 70 °C, as well as at a pH below 7 and above 8. This indicated that the bacteria can be identified as moderate thermophiles and neutrophils and can potentially serve as a source of thermostable and alkaline enzymes.

## CONCLUSIONS

1. Thermophilic bacteria from different layers of a woody-chip pile were isolated. A total of 18 bacterial strains were isolated, identified, and characterized.
2. Preliminary activity tests revealed that all 18 strains were capable of producing both amylases and xylanases.
3. The strains 7 and 16 found in the bark layer of the woody pile showed the highest xylanase activity (4.4 and 5.4 U/mL) and were studied in detail. It was established that those strains were moderate thermophiles and neutrophils with a temperature and pH optimum ranging from 57 to 60 °C and 7.0 to 8.5, respectively.
4. The study also evaluated the effect of various carbon substrates on the xylanase activity. It has been shown that the birch chips and brewers' spent grain have potential to be economically viable carbon substrates for xylanase production by *P. thermoglucosidasius* and *P. caldxylosilyticus* bacterial strains.

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